Maximum standardized uptake value in ^{18}F-fluoro-2-deoxyglucose positron emission tomography is associated with advanced tumor factors in esophageal cancer

TAKESHI KAJIWARA[1], YOICHI HIASA[2], TOMOHIRO NISHINA[1], TOSHIHIKO MATSUMOTO[1], SHINICHIRO HORI[1], SEIJIN NADANO[1], HARUO IGUCHI[1], SATORU TAKEJI[2], EIJI TSUBOUCHI[2], YOSHIO IKEDA[2] and MORIKAZU ONJI[2]

[1]Department of Gastroenterology, National Hospital Organization Shikoku Cancer Center, Matsuyama, Ehime 791-0280; [2]Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Toon, Ehime 791-0295, Japan

Received May 24, 2013; Accepted December 19, 2013

DOI: 10.3892/mco.2014.238

Abstract. Positron emission tomography/computed tomography (PET/CT) with ^{18}F-fluoro-2-deoxyglucose (FDG-PET/CT) has become established in cancer imaging, and derived maximum standardized uptake values (SUVmax) add functional information regarding cancer, including esophageal squamous cell carcinoma (ESCC). The aim of the present study was to determine the clinical significance and association of tumor progression using SUVmax derived from PET/CT images in patients with ESCC. In total, 101 patients with ESCC were assessed using FDG-PET/CT and the SUVmax was then compared with the clinical backgrounds and prognoses of the patients. Endoscopic ESCC biopsy specimens were obtained in order to analyze mRNA expression relative to tumor progression. The results showed that values for SUVmax were significantly higher in patients with tumor progression factors, particularly those with lymph node metastasis. Analysis of receiver operating characteristics curves revealed an optimum SUVmax cut-off value of 10.26 for node-positive disease. Patients with SUVmax ≥10.26 had gene alterations with epithelial-mesenchymal transition (EMT) and significantly worse overall survival (P=0.0012). A higher SUVmax in patients with ESCC was associated with lymph node metastasis and a poorer prognosis. Thus, the SUVmax may reflect the potential of EMT in patients with ESCC.

Correspondence to: Professor Yoichi Hiasa, Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Shitsukawa, Toon, Ehime 791-0295, Japan
E-mail: hiasa@m.ehime-u.ac.jp

Abbreviations: BSC, best supportive care; CI, confidence interval; CYFRA, cytokeratin 19 fragment; ECOG PS, Eastern Cooperative Oncology Group performance status; EMT, epithelial-mesenchymal transition; ESCC, esophageal squamous cell carcinoma; FDG, ^{18}F-fluoro-2-deoxyglucose; FN, fibronectin; FP, chemotherapy of 5-fluorouracil plus cisplatin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporter; IL, interleukin; MMP, matrix metalloproteinase; OS, overall survival; PDGFR, platelet-derived growth factor receptor; PET/CT, positron emission tomography/computed tomography; RT, radiotherapy; RT-PCR, reverse transcription polymerase chain reaction; SCC, squamous cell carcinoma; SE, standard error; SUVmax, maximum standardized uptake value; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; TNM, tumor-node-metastasis; UICC, Union for International Cancer Control

Key words: esophageal squamous cell carcinoma, ^{18}F-fluoro-2-deoxyglucose, positron emission tomography, maximum standardized uptake value, epithelial-mesenchymal transition

Introduction

Esophageal cancer is an extremely lethal gastrointestinal neoplasm that leads to >300,000 mortalities worldwide annually (1). The reason for the poor prognosis is that >50% of patients already have unresectable or metastatic disease when they are diagnosed with esophageal cancer (2). A precise evaluation of the prognosis or overall survival (OS) of patients with esophageal cancer is essential for selecting appropriate treatment. Positron emission tomography/computed tomography (PET/CT) has become established in cancer imaging, and is useful for stratification during the primary staging of esophageal cancer by anatomical factors, such as tumor depth, invasion, lymph node metastasis and distant metastasis (3-10). However, ^{18}F-fluoro-2-deoxyglucose (FDG)-PET may be used to evaluate functional factors associated with tumor activity, which depends on glucose metabolism.

It has been previously reported that the maximum standardized uptake value (SUVmax) in patients with certain types of cancer significantly correlates with survival, prognosis and recurrence (11,12). Although the clinical significance of SUVmax in esophageal cancer has been reported (13,14), the progression of esophageal cancer in specimens of esophageal squamous cell carcinoma (ESCC) using gene analysis has not yet been assessed. Therefore we investigated whether the

SUVmax derived from FDG-PET/CT is associated with tumor progression and prognosis. We also compared gene alterations in ESCC biopsy specimens with SUVmax to determine factors that are associated with tumor progression and prognosis.

Materials and methods

Patients. Biopsy specimens of ESCC were obtained from 101 treatment-naïve patients who underwent endoscopy between March 2007 and January 2010 at the National Hospital Organization Shikoku Cancer Center. The patients had not undergone prior endoscopic mucosal resection, chemotherapy, radiotherapy or surgery and had no other active malignancies. Pretreatment tumor specimens were collected by endoscopic biopsy subsequent to obtaining written informed consent from each patient. These and all other specimens were histologically proven as ESCC. The patients were assessed using FDG-PET/CT, and then the SUVmax for each primary tumor was calculated. Tumors were clinically staged according to the criteria of the tumor-node-metastasis (TNM) classification of the International Union Against Cancer (15). The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, and the Institutional Review Board of the National Hospital Organization Shikoku Cancer Center approved this study (approval no. H18-34).

FDG-PET/CT imaging. Patients were examined by PET/CT imaging using Aquiduo (Toshiba Medical Systems Corporation, Otawara, Japan), which provides separate CT and PET datasets that can be accurately combined on a computer workstation. Whole-body CT [Auto-mA (SDN), 120 kV, 2.0 mm x 16, 0.5 sec, 30 mm/rotation (HP15), 2- and 4-mm incremental reconstructions] covered the region from the head to the upper femoral regions. The PET component of the combined imaging system has an axial field of view of 16.2 cm (per bed position) with an in-plane spatial resolution of 4.6 mm. PET images in the same field of view as the CT were obtained over a period of 90 min following the administration of 3.0 MBq/kg body weight of FDG. The duration of PET image acquisition was adapted according to the weight of each patient. Images were scatter-corrected and reconstructed with and without PET attenuation correction, which was based on the CT data. Prior to injecting the radioactive tracer, blood was sampled to ensure that blood glucose levels were within the normal range. The single-pixel SUVmax normalized using lean body mass was quantified in the primary tumor when uptake was abnormal.

Polymerase chain reaction (PCR) array analysis. Cancer-related genes from ESCC specimens were comprehensively analyzed using RT2 Profiler PCR array systems for Cancer Pathway Finder and the epithelial-mesenchymal transition (EMT) signaling pathway (Qiagen, Tokyo, Japan), as well as the LightCycler system (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Threshold cycle values were analyzed using web-based PCR array data analysis software (http://www.sabiosciences.com/pcr/arrayanalysis.php). Prior to data application, we confirmed that the reverse transcriptase, cDNA and positive PCR controls were within acceptable ranges. Three samples each with the highest and lowest SUVmax were analyzed. The same amount of genes (0.5 μg per sample) was mixed with the six samples and then the analysis proceeded using the PCR array systems.

Extraction of RNA and quantitative PCR (qPCR). Esophageal samples collected by endoscopic biopsy were immediately transferred to RNAlater (Life Technologies, Grand Island, NY, USA) and homogenized. Total RNA was extracted using the RNeasy Mini kit (Qiagen) and reverse-transcribed using an oligo d(T)16 primer and RT-PCR kits (Applied Biosystems, Foster City, CA, USA) under standard conditions. qPCR amplification proceeded using a LightCycler system (Roche Diagnostics) and SYBR-Green I dye (Roche Diagnostics) with commercially available primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), collagen-1α2, E-cadherin, fibronectin (FN)-1, interleukin (IL)-8, matrix metalloproteinase (MMP)-1, MMP-2, MMP-3, MMP-9, Snai-1 (Snail), transforming growth factor (TGF)-β1, TGF-β2, TGF-β3, tissue inhibitor of metalloproteinases (TIMP)-1, tumor necrosis factor (TNF)-α and Twist-1 primer sets (Roche Search LC, Heidelberg, Germany). For other genes, *Taq*Man real-time PCR systems (Life Technologies) with commercial integrin-α5, platelet-derived growth factor receptor (PDGFR)-B, Snai-2 (Slug), WNT-11, N-cadherin, vimentin and glucose transporter (GLUT)-1 (Life Technologies) was used according to the manufacturer's instructions. The thermal cycling program comprised 40 cycles of 95˚C for 10 sec, 62˚C for 10 sec and 72˚C for 15 sec using the LightCycler and 40 cycles of 95˚C for 15 sec, 60˚C for 60 sec using the *Taq*Man real-time PCR systems. The PCR efficiency for the mRNA of these genes was measured from standard curves generated by serial dilution of the cDNA for the LightCycler, and the ΔCT values were determined for the *Taq*Man real-time PCR systems. The relative expression of these genes was compared with the housekeeping control gene, GAPDH. Data were normalized for GAPDH expression using a comparative threshold cycle method. The relative mRNA expression levels divided by the amount of GAPDH mRNA were statistically evaluated.

Statistical analysis. Data were statistically analyzed using JMP 9.0 software (SAS Institute, Cary, NC, USA). Associations between SUVmax and clinical variables as well as mRNA expression were evaluated using the Wilcoxon rank-sum test for significance. Independent variables contributing to SUVmax were evaluated by linear regression analysis and included in the multivariate analysis. To stratify patients with high and low SUVmax, the optimum cut-off value was defined as the point of the receiver operating characteristic curve with the maximum Youden index: sensitivity + specificity -100% (16). Survival curves were generated using the Kaplan-Meier method, and statistical differences between curves were calculated using the log-rank test. Statistical significance was defined as $P<0.05$ based on a two-tailed test.

Results

Patient characteristics. Table I shows the characteristics of the 101 patients who were recently diagnosed with ESCC. The median age was 63 years (range, 47-88) and 90.1% of

Table I. Patients' characteristics.

Characteristics	Value
No. of patients	101
Age (years)	
Median	63
Range	47-88
Gender	
Male	91
Female	10
ECOG PS	
0	88
1	11
2	2
Tumor location	
Upper	18
Middle	53
Lower	30
Tumor size (longest diameter, cm)	
Median	6.0
Range	1.5-35.0
T stage	
T1	13
T2	39
T3	26
T4	23
N stage	
N0	34
N1	67
M stage	
M0	73
M1	28
Clinical stage (UICC)	
I	11
II	33
III	29
IV	28
SCC antigen (ng/ml) (n=100)	
Median	1.6
Range	0.2-31.3
CYFRA (ng/ml) (n=84)	
Median	2.25
Range	0.8-36.7
SUVmax	
Median	13.64
Range	2.1-31.99
Treatment	
FP+radiotherapy	60
Surgery after neoadjuvant FP	20
Surgery	9
FP	6
Radiotherapy	3
Best supportive care	3

ECOG PS, Eastern Cooperative Oncology Group performance status; UICC, Union for International Cancer Control; SCC, squamous cell carcinoma; CYFRA, cytokeratin 19 fragment; SUVmax, maximal standardized uptake values; FP, 5-fluorouracil plus cisplatin.

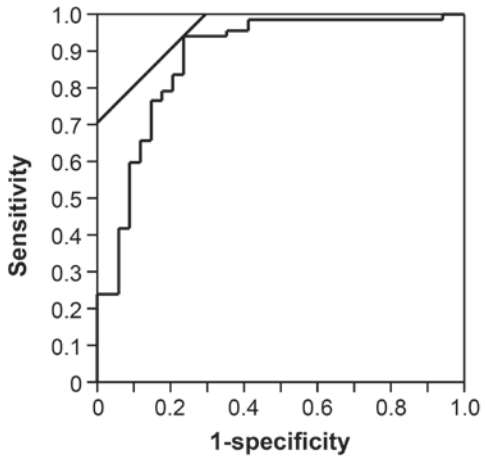

Figure 1. Receiver operating characteristics curve for N stage and maximum standardized uptake value (SUVmax). Optimum cut-off value for SUVmax for differentiating a high and low SUVmax was determined by analyzing receiver operating characteristics curves for N stage. Optimum SUVmax cut-off value that differentiated a positive and negative tumor metastasis to lymph nodes was 10.26 with 94.0%, sensitivity and 76.5% specificity.

the patients were male. ESCC sites were comparable to those reported in Japan (17). The median value of the longest diameter of ESCC was 6.0 cm (range, 1.5-35.0). With respect to clinical TNM staging, 67 (66.3%) patients were node-positive and 28 (27.7%) had distant metastasis. The levels of the tumor markers, squamous cell carcinoma (SCC) antigen and cytokeratin 19 fragment (CYFRA) were examined. The median value of SCC antigen was 1.6 ng/ml (range, 0.2-31.3), and that of CYFRA was 2.25 ng/ml (range, 0.8-36.7). The median SUVmax determined by FDG-PET/CT was 13.64 (range, 2.1-31.99). Table I shows administration of treatment to patients.

Univariate analysis of clinical variables and SUVmax. Table II shows the associations between clinical variables and SUVmax as determined by univariate analysis. The SUVmax was significantly higher in patients with tumors ≥6 cm (P<0.0001), T3 or T4 disease (P<0.0001), N1 disease (P<0.0001), M1 disease (P=0.0424), clinical stages III or IV disease (P<0.0001) and high (>2 ng/ml) SCC antigen levels (P=0.0002). The SUVmax did not significantly differ for any other clinical variables.

Multivariate analysis of independent variables contributing to the SUVmax of primary ESCC tumors. Table III shows the results of the multivariate analysis that included tumor size, T stage, N stage, M stage, clinical stage and SCC antigen as variables that significantly differed in the univariate analysis. Among them, N stage was the most significantly associated [regression coefficient, 3.63; 95% confidence interval (CI), 2.26-5.00; P<0.0001] with SUVmax, followed by SCC antigen levels (regression coefficient, 1.20; 95% CI, 0.200-2.21; P=0.0192).

Expression of cancer-related genes and GLUT-1 in patients with ESCC. Receiver operating characteristics curves for the N stage were analyzed to determine the optimum cut-off value in order to differentiate groups with a high and low SUVmax.

Table II. Univariate analysis of SUVmax.

Variable	No.	Median (range) SUVmax	P-value[a]
Age (years)			
<65	55	13.0 (2.10-23.8)	0.189
≥65	46	14.6 (2.37-32.0)	
Gender			
Male	91	14.1 (2.10-32.0)	0.174
Female	10	11.0 (4.74-17.7)	
ECOG PS			
0	88	13.3 (2.10-32.0)	0.0933
1-2	13	17.7 (2.37-22.7)	
Tumor size (cm)			
<6	50	10.6 (2.10-25.4)	<0.0001
≥6	51	15.6 (3.54-32.0)	
T stage			
T1/2	52	10.3 (2.10-32.0)	<0.0001
T3/4	49	15.7 (2.37-24.6)	
N stage			
N0	34	5.98 (2.10-19.1)	<0.0001
N1	67	15.6 (2.37-32.0)	
M stage			
M0	73	12.6 (2.10-32.0)	0.0424
M1	28	14.3 (8.14-24.6)	
Clinical stage (UICC)			
Stage I/II	44	9.31 (2.10-32.0)	<0.0001
Stage III/IV	57	15.4 (2.37-24.6)	
SCC antigen (ng/ml)			
Normal (≤2)	63	11.8 (2.10-32.0)	0.0002
High (>2)	37	15.6 (5.00-25.4)	
CYFRA (ng/ml)			
Normal (≤3.5)	63	12.6 (2.10-32.0)	0.252
High (>3.5)	21	14.4 (2.70-24.3)	

[a]Analyzed by the Wilcoxon rank-sum test. SUVmax, maximal standardized uptake values; ECOG PS, Eastern Cooperative Oncology Group performance status; UICC, Union for International Cancer Control; SCC, squamous cell carcinoma; CYFRA, cytokeratin 19 fragment.

Table III. Multivariate analysis of SUVmax.

Variable	Regression coefficient	SE	95% CI	P-value
Tumor size	0.99	0.521	-0.0419-2.03	0.0598
T stage	1.26	0.719	-0.163-2.69	0.0819
N stage	3.63	0.689	2.26-5.00	<0.0001
M stage	0.04	0.681	-1.32-1.39	0.958
Clinical stage (UICC)	-1.12	0.993	-3.09-0.852	0.263
SCC antigen	1.20	0.505	0.200-2.21	0.0192

SUVmax, maximal standardized uptake values; SE, standard error; CI, confidence interval; UICC, Union for International Cancer Control; SCC, squamous cell carcinoma.

The optimum SUVmax cut-off for discriminating positive and negative tumor metastasis to lymph nodes was 10.26 (Fig. 1). The sensitivity was 94.0% and the specificity was 76.5%.

Expression of cancer-related genes and GLUT-1 (a key FDG transporter in cancer cells) was compared between patients with high and low SUVmax. We comprehensively

Table IV. PCR array for cancer pathway-related genes.

Gene	Fold difference: High SUVmax (n=3)/ low SUVmax (n=3)	Fold up- or downregulation: High SUVmax (n=3)/ low SUVmax (n=3)
AKT1	0.63	-1.59
ANGPT1	1.22	1.22
ANGPT2	**3.40**	**3.40**
APAF1	0.56	-1.77
ATM	0.54	-1.84
BAD	0.68	-1.46
BAX	0.63	-1.59
BCL2	**0.18**	**-5.49**
BCL2L1	0.63	-1.58
BRCA1	0.50	-1.99
CASP8	0.73	-1.36
CCNE1	0.63	-1.59
CDC25A	1.14	1.14
CDK2	0.85	-1.18
CDK4	0.50	-1.98
CDKN1A	1.47	1.47
CDKN2A	0.67	-1.49
CFLAR	0.58	-1.71
CHEK2	**0.33**	**-3.00**
COL18A1	1.56	1.56
E2F1	0.51	-1.97
ERBB2	0.68	-1.48
ETS2	0.52	-1.93
FAS	**0.44**	**-2.26**
FGFR2	**0.26**	**-3.88**
FOS	0.87	-1.15
GZMA	1.91	1.91
HTATIP2	**0.48**	**-2.06**
IFNA1	1.30	1.30
IFNB1	0.51	-1.97
IGF1	0.63	-1.59
IL8	**6.04**	**6.04**
ITGA1	1.60	1.60
ITGA2	0.65	-1.54
ITGA3	**2.03**	**2.03**
ITGA4	0.66	-1.51
ITGAV	**2.02**	**2.02**
ITGB1	**2.18**	**2.18**
ITGB3	**0.46**	**-2.17**
ITGB5	**0.44**	**-2.26**
JUN	**0.01**	**-192.14**
MAP2K1	0.78	-1.28
MCAM	1.22	1.22
MDM2	0.51	-1.97
MET	0.96	-1.05
MMP1	**21.47**	**21.47**
MMP2	**2.32**	**2.32**
MMP9	**2.66**	**2.66**
MTA1	1.52	1.52
MTA2	0.84	-1.19
MTSS1	1.82	1.82
MYC	0.86	-1.16

Table IV. Continued.

Gene	Fold difference: High SUVmax (n=3)/ low SUVmax (n=3)	Fold up- or downregulation: High SUVmax (n=3)/ low SUVmax (n=3
MYC	0.86	-1.16
NFKB1	0.69	-1.45
NFKBIA	1.83	1.83
NME1	0.52	-1.94
NME4	0.63	-1.59
PDGFA	1.04	1.04
PDGFB	0.94	-1.06
PIK3R1	**0.24**	**-4.22**
PLAU	**3.17**	**3.17**
PLAUR	**6.70**	**6.70**
PNN	1.03	1.03
RAF1	0.59	-1.69
RB1	0.64	-1.56
S100A4	1.51	1.51
SERPINB5	0.53	-1.90
SERPINE1	**5.26**	**5.26**
SNCG	**0.35**	**-2.84**
SYK	**0.09**	**-11.13**
TEK	0.52	-1.94
TERT	0.63	-1.58
TGFB1	0.65	-1.54
TGFBR1	1.47	1.47
THBS1	**3.24**	**3.24**
TIMP1	**2.27**	**2.27**
TIMP3	**0.48**	**-2.08**
TNF	**4.24**	**4.24**
TNFRSF10B	1.00	1.00
TNFRSF1A	0.77	-1.30
TNFRSF25	0.99	-1.01
TP53	**0.39**	**-2.60**
TWIST1	0.95	-1.05
EPDR1	1.50	1.50
VEGFA	0.98	-1.03
B2M	**2.57**	**2.57**
HPRT1	1.36	1.36
RPL13A	**0.44**	**-2.28**
GAPDH	0.62	-1.61
ACTB	1.05	1.05

Genes indicated in bold type were up- or downregulated by a difference of >2-fold between the two groups.

analyzed which cancer-related genes were associated with SUVmax using a PCR array for the cancer pathway (Table IV) and mRNA (0.5 μg) in biopsy specimens from the three patients each with the highest and lowest SUVmax. Genes related to the EMT signaling pathway (integrins and MMPs) were expressed at 2-fold higher levels in the three patients with the lowest SUVmax. Therefore genes involved in the EMT signaling pathway were analyzed using a PCR array

Table V. PCR array for epithelial-mesenchymal transition (EMT) related genes.

Gene	Fold difference: High SUVmax (n=3)/ low SUVmax (n=3)	Fold up- or downregulation: High SUVmax (n=3)/ low SUVmax (n=3)
AHNAK	0.77	-1.30
AKT1	0.84	-1.18
BMP1	0.94	-1.06
BMP7	**0.46**	**-2.19**
CALD1	**2.28**	**2.28**
CAMK2N1	**2.52**	**2.52**
CAV2	1.45	1.45
CDH1 (E-cadherin)	0.65	-1.54
CDH2 (N-cadherin)	1.47	1.47
COL1A2 (Collagen-1α2)	**11.84**	**11.84**
COL3A1	**7.65**	**7.65**
COL5A2	**5.96**	**5.96**
CTNNB1	**0.50**	**-2.02**
DSC2	0.58	-1.73
DSP	**0.48**	**-2.06**
EGFR	**0.22**	**-4.45**
ERBB3	**0.43**	**-2.30**
ESR1	0.80	-1.24
F11R	0.62	-1.62
FGFBP1	1.17	1.17
FN1	**17.83**	**17.83**
FOXC2	1.84	1.84
FZD7	**0.10**	**-10.30**
GNG11	0.96	-1.05
GSC	**0.32**	**-3.08**
GSK3B	**0.44**	**-2.27**
IGFBP4	1.22	1.22
IL1RN	1.54	1.54
ILK	1.11	1.11
ITGA5 (Integrin-α5)	**5.45**	**5.45**
ITGAV	1.34	1.34
ITGB1	1.81	1.81
JAG1	1.04	1.04
KRT14	1.80	1.80
KRT19	**0.41**	**-2.45**
KRT7	1.15	1.15
MAP1B	**0.34**	**-2.92**
MITF	1.22	1.22
MMP2	**4.33**	**4.33**
MMP3	**3.40**	**3.40**
MMP9	**4.37**	**4.37**
MSN	1.62	1.62
MST1R	1.40	1.40
NODAL	**0.34**	**-2.92**
NOTCH1	0.58	-1.73
NUDT13	0.60	-1.66
OCLN	0.66	-1.52
PDGFRB	**5.16**	**5.16**
PLEK2	**4.52**	**4.52**
PPPDE2	0.56	-1.77
PTK2	0.71	-1.40
PTP4A1	0.80	-1.24
RAC1	0.75	-1.33
RGS2	1.22	1.22
SERPINE1	**5.96**	**5.96**
SIP1	1.53	1.53
SMAD2	**0.39**	**-2.59**
SNAI1 (Snail)	**2.94**	**2.94**
SNAI2 (Slug)	1.35	1.35
SNAI3	0.77	-1.30
SOX10	0.65	-1.53
SPARC	**5.16**	**5.16**
SPP1	0.53	-1.87
STAT3	0.80	-1.25
STEAP1	1.85	1.85
TCF3	0.60	-1.67
TCF4	**0.38**	**-2.61**
TFPI2	**2.84**	**2.84**
TGFB1	1.28	1.28
TGFB2	1.14	1.14
TGFB3	**2.78**	**2.78**
TIMP1	**2.51**	**2.51**
TMEFF1	0.66	-1.52
TMEM132A	1.07	1.07
TSPAN13	1.80	1.80
TWIST1	**2.23**	**2.23**
VCAN	**5.30**	**5.30**
VIM (Vimentin)	**2.05**	**2.05**
VPS13A	0.55	-1.81
WNT11	**5.12**	**5.12**
WNT5A	0.67	-1.49
WNT5B	**2.96**	**2.96**
ZEB1	1.01	1.01
ZEB2	1.33	1.33
B2M	1.99	1.99
HPRT1	1.39	1.39
RPL13A	**0.48**	**-2.09**
GAPDH	0.66	-1.51
ACTB	1.14	1.14

Genes indicated in bold type were up- or downregulated by a difference of >2-fold between the two groups.

(Table V). Table VI shows the 21 cancer- or EMT-related genes that required additional investigation. To confirm differences between a high and low SUVmax, mRNA isolated from the ESCC of all 101 patients was analyzed using qPCR and

Table VI. Gene expression in groups with low and high SUVmax.

Gene	Median (range) mRNA expression levels		P-value[a]
	Low SUVmax (n=30)	High SUVmax (n=71)	
Collagen-1α2	8.55E-2 (2.43E-3-1.37)	1.99E-1 (3.29E-3-2.19)	0.0129
E-cadherin	8.14E-3 (1.87E-3-1.49E-2)	4.70E-3 (4.05E-5-3.08E-2)	0.0351
FN-1	1.12E-3 (1.95E-5-3.54E-2)	3.99E-3 (2.20E-5-5.88E-2)	0.0032
IL-8	3.76E-2 (1.78E-3-6.52E-1)	1.27E-1 (7.04E-3-2.18)	0.0042
Integrin-α5	1.19E-3 (1.88E-4-8.53E-3)	2.23E-3 (3.98E-4-1.57E-2)	0.0008
MMP-1	1.18E-2 (4.50E-5-1.86E-1)	2.61E-2 (1.48E-3-5.30E-1)	0.0263
MMP-2	7.30E-3 (3.58E-4-2.86E-1)	2.61E-2 (8.14E-4-5.87E-1)	0.0286
MMP-3	4.24E-2 (8.06E-5-6.21E-1)	5.91E-2 (3.69E-4-8.43E-1)	0.123
MMP-9	1.99E-2 (2.11E-3-1.31E-1)	1.95E-2 (2.04E-3-2.30E-1)	0.611
N-cadherin	2.98E-6 (1.29E-7-1.06E-4)	7.81E-6 (7.05E-8-4.97E-4)	0.0172
PDGFR-B	1.06E-4 (4.95E-6-8.15E-4)	1.82E-4 (1.69E-6-1.44E-3)	0.0703
Snail	3.68E-4 (3.79E-5-2.70E-3)	6.69E-4 (1.76E-4-4.53E-3)	0.0059
Slug	8.15E-4 (7.92E-5-2.26E-3)	7.79E-4 (3.20E-5-4.74E-3)	0.798
TGF-β1	1.82E-2 (6.37E-3-8.25E-2)	1.82E-2 (4.23E-3-7.00E-2)	0.385
TGF-β2	4.38E-4 (5.08E-5-2.45E-3)	4.32E-4 (2.65E-5-3.53E-3)	0.873
TGF-β3	1.78E-4 (6.11E-5-1.51E-3)	3.41E-4 (1.32E-6-2.21E-3)	0.0895
TIMP-1	6.59E-2 (1.50E-2-4.16E-1)	1.04E-1 (1.31E-2-1.77)	0.0165
TNF-α	1.53E-3 (4.19E-4-1.34E-2)	1.36E-3 (2.79E-4-2.58E-2)	0.956
Twist-1	3.46E-3 (5.50E-4-1.94E-2)	4.58E-3 (5.35E-4-2.21E-2)	0.0801
Vimentin	8.76E-3 (1.99E-3-5.30E-2)	1.01E-2 (6.00E-5-7.55E-2)	0.109
WNT-11	9.08E-6 (7.18E-7-7.86E-4)	8.73E-6 (2.72E-8-9.83E-4)	0.781
GLUT-1	9.64E-3 (2.96E-3-3.35E-2)	9.58E-3 (2.18E-3-7.81E-2)	0.675

[a]Analyzed by Wilcoxon rank-sum test. SUVmax, maximal standardized uptake values; FN, fibronectin; IL, interleukin; MMP, matrix metalloproteinase; PDGFR, platelet-derived growth factor receptor; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; GLUT, glucose transporter.

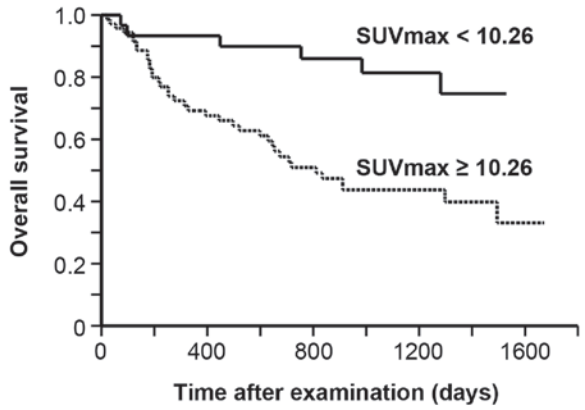

Figure 2. Survival curves for groups with high and low maximum standardized uptake values (SUVmax). Survival rates are significantly poorer in the group with a higher SUVmax compared with the group with a lower SUVmax. Overall survival (OS) did not reach median in patients with a low SUVmax, whereas median OS was 27.0 months in those with a high SUVmax (hazard ratio, 3.77; 95% confidence interval, 1.71-9.94; P=0.0012).

gene-specific primers (Table VI). The mRNA expression of collagen-1α2, FN-1, IL-8, integrin-α5, MMP-1, MMP-2, N-cadherin, Snail and TIMP-1 was significantly higher in the group above, than that below the SUVmax cut-off value of 10.26 (P=0.0129, 0.0032, 0.0042, 0.0008, 0.0263, 0.0286, 0.0172, 0.0059 and 0.0165, respectively). E-cadherin mRNA expression was significantly lower in the group with a high compared with a low SUVmax (P=0.0351). Differences in these genes were compatible with EMT induction and with an increased SUVmax in ESCC. However, GLUT-1 mRNA expression did not significantly differ between the groups with a high and low SUVmax.

Levels of SUVmax are associated with OS. We analyzed the prognosis of patients with SUVmax above and below the 10.26 cut-off value. The median follow-up duration of all the patients was 26.5 months (range, 0.67-55.8). The median OS of all patients was 49.8 months, and the 3-year survival rate was 55.3%. The survival rate was significantly poorer in the group with a higher SUVmax (Fig. 2). The median OS of patients with a high SUVmax was 27.0 months (hazard ratio, 3.77; 95% CI, 1.71-9.94; P=0.0012), whereas that of patients with a low SUVmax did not reach the median.

Discussion

In the present study, we analyzed the association between clinical variables and SUVmax derived from FDG-PET/CT at the time of initial diagnosis. We also examined the mRNA levels of cancer-related genes from biopsy specimens of ESCC to compare tumor progression with SUVmax. The SUVmax

was associated with tumor progression factors, particularly with lymph node metastasis. A higher SUVmax is associated with modulated genes involved in EMT formation, which would be linked to an advanced clinical N stage.

The current TNM classification for esophageal cancer is based only on anatomical factors as compared to functional factors such as FDG uptake (15). However, mounting evidence suggests that functional factors are involved in tumor progression and prognosis, although possibly not to a greater extent than anatomical factors in esophageal cancer (3-10). Non-invasive FDG-PET/CT may aid in the detection of not only anatomical, but also some genetic, oncological, molecular and biological factors. Two studies that have examined the SUVmax of FDG-PET have also provided staging, biological and prognostic information regarding esophageal cancer (13,14). By contrast, other authors have reported that SUVmax is not a useful independent predictor of survival for patients with esophageal cancer (18,19). Those studies found that SUVmax correlates with TNM classification. Therefore, we considered that SUVmax is a parameter of tumor progression in ESCC. To the best of our knowledge, this is the first demonstration of the N stage being the most significant variable that contributes to the SUVmax of primary tumors, although two previous studies have identified FDG-positive lymph nodes as the most significant risk factor for recurrence and a predictor of poor outcome (14,20). Additional analysis is required to confirm the clinical implications of SUVmax in ESCC with lymph node metastasis.

EMT is essential for morphogenesis during embryonic development and is triggered during carcinoma progression to assume an invasive and metastatic state (21,22). The EMT may be reactivated in a variety of diseases, including fibrosis and cancer progression (23). Growth factors, cytokines, extracellular matrix components and transcription factors are involved in EMT induction in epithelial tumor cells (21,22). Results of recent studies (24-29) suggest that EMT is associated with tumor invasion, metastasis and prognosis in ESCC. However, an association between SUVmax derived from FDG-PET/CT and EMT markers in ESCC remains to be determined. In the present study, we used qPCR to measure EMT marker expression in pretreated endoscopic esophageal tumor biopsy specimens. Among the EMT markers, we found that the expression of Snail, collagen-1α2, E-cadherin, FN-1, integrin-α5, MMP-1, MMP-2, N-cadherin, TIMP-1 and IL-8 significantly differed according to SUVmax in patients with ESCC. Additinoally, the transcription factor (Snail), extracellular matrix and cell adhesion factors (collagen-1α2, E-cadherin, FN-1, integrin-α5, MMP-1, MMP-2, N-cadherin and TIMP-1) and an angiogenesis factor (IL-8) contributed to the promotion of EMT (21-23,30).

E-cadherin is important in the regulation of intercellular adhesion in normal cell structures, as well as in cancer invasion and metastasis. Low E-cadherin expression was significantly associated with a high SUVmax in the present study. Previously, it was reported that reduced E-cadherin expression is associated with tumor invasion, metastasis and a poor prognosis for patients with ESCC (24,25). Our results are comparable with those findings. The direct transcriptional repression of E-cadherin results in the induction of EMT by Snail in epithelial cells. Therefore, Snail is important in the migration and invasive activity by repressing epithelial adhesion molecules, which contributes to deeper invasion, metastasis and a poor prognosis (24,26). Integrin promotes Snail expression through integrin-linked kinase activation (24). MMP-2 expression, which is crucial in extracellular matrix remodelling, is significantly associated with tumor invasion and metastasis in ESCC (27). TIMP-1 both inhibits MMPs and is crucial as a growth factor. The expression of TIMP-1 is associated with tumor progression and a poor prognosis (28). N-cadherin is upregulated in invasive tumors, plays a key role in intercellular adhesion and is an important factor in ESCC tumor progression (29).

The SUVmax in ESCC significantly correlates with the expression of GLUT-1, which is a GLUT (31). However, we did not find a significant difference in GLUT-1 expression between a high and low SUVmax. We hypothesize that the tumor glucose metabolism is connected with EMT. Wnt/Snail signaling which inhibits mitochondrial respiration and induces glycolytic changes. The EMT may be a contributary factor to the Wnt/Snail regulation of mitochondrial function and glucose metabolism (32). However, details of the molecular mechanisms of FDG uptake in tumors remain controversial. Assessments of FDG uptake during the EMT are therefore required.

Limitations of the present study should be considered. The original endoscopic biopsy specimens were small, and they might not have been representative of entire tumors due to intratumor heterogeneity. However, biopsy material frequently yields a representative genetic expression profile of total tumor tissue (33), and biopsy specimens are the only tissue samples that can be conveniently obtained for this type of study. The second limitation is the definition of the optimum SUVmax cut-off because it depends on the method and/or FDG-PET/CT scanners. The significance of our cut-off SUVmax for prognosis may require re-evaluation at each institution. The third is that the patients had different stages of disease and underwent different therapies, which affect prognosis. However, the univariate analysis of OS revealed that the SUVmax derived from FDG-PET/CT was associated with the prognosis of patients with esophageal cancer.

In conclusion, findings of the present study have shown that a higher SUVmax derived from FDG-PET/CT is associated with lymph node metastasis and a poorer prognosis for patients with ESCC. Furthermore, tumor functional analysis at a higher SUVmax indicated the EMT tendency of ESCC. The usefulness of SUVmax should be validated in a clinical study.

Acknowledgements

This study was supported in part by a Grant-in-Aid for Scientific Research (Japan Society for the Promotion of Science, KAKENHI 24590980, to Y.H.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, partially by a Grant-in-Aid for Scientific Research and Development (to Y.H.), and by the National Cancer Center Research and Development Fund (23-A-14, to T.K.) from the Japanese Ministry of Health, Labor and Welfare, Japan. The authors are grateful to Ms. Yumi Osada (National Hospital Organization Shikoku Cancer Center), Ms. Chie Takeichi and Ms. Sakiko Inoh

(Ehime University Graduate School of Medicine) for valuable technical assistance.

References

1. Parkin DM, Bray FI and Devesa SS: Cancer burden in the year 2000. The global picture. Eur J Cancer 37 Suppl 8: S4-S66, 2001.
2. Enzinger PC and Mayer RJ: Esophageal cancer. N Engl J Med 349: 2241-2252, 2003.
3. Barber TW, Duong CP, Leong T, Bressel M, Drummond EG and Hicks RJ: 18F-FDG PET/CT has a high impact on patient management and provides powerful prognostic stratification in the primary staging of esophageal cancer: a prospective study with mature survival data. J Nucl Med 53: 864-871, 2012.
4. Bar-Shalom R, Guralnik L, Tsalic M, Leiderman M, Frenkel A, Gaitini D, Ben-Nun A, Keidar Z and Israel O: The additional value of PET/CT over PET in FDG imaging of oesophageal cancer. Eur J Nucl Med Mol Imaging 32: 918-924, 2005.
5. Berrisford RG, Wong WL, Day D, Toy E, Napier M, Mitchell K and Wajed S: The decision to operate: role of integrated computed tomography positron emission tomography in staging oesophageal and oesophagogastric junction cancer by the multidisciplinary team. Eur J Cardiothorac Surg 33: 1112-1116, 2008.
6. Kato H, Kimura H, Nakajima M, Sakai M, Sano A, Tanaka N, Inose T, Faried A, Saito K, Ieta K, Sohda M, Fukai Y, Miyazaki T, Masuda N, Fukuchi M, Ojima H, Tsukada K, Oriuchi N, Endo K and Kuwano H: The additional value of integrated PET/CT over PET in initial lymph node staging of esophageal cancer. Oncol Rep 20: 857-862, 2008.
7. Noble F, Bailey D; SWCIS Upper Gastrointestinal Tumour Panel, Tung K and Byrne JP: Impact of integrated PET/CT in the staging of oesophageal cancer: a UK population-based cohort study. Clin Radiol 64: 699-705, 2009.
8. Omloo JM, van Heijl M, Hoekstra OS, van Berge Henegouwen MI, van Lanschot JJ and Sloof GW: FDG-PET parameters as prognostic factor in esophageal cancer patients: a review. Ann Surg Oncol 18: 3338-3352, 2011.
9. Salahudeen HM, Balan A, Naik K, Mirsadraee S and Scarsbrook AF: Impact of the introduction of integrated PET-CT into the preoperative staging pathway of patients with potentially operable oesophageal carcinoma. Clin Radiol 63: 765-773, 2008.
10. Yuan S, Yu Y, Chao KS, Fu Z, Yin Y, Liu T, Chen S, Yang X, Yang G, Guo H and Yu J: Additional value of PET/CT over PET in assessment of locoregional lymph nodes in thoracic esophageal squamous cell cancer. J Nucl Med 47: 1255-1259, 2006.
11. Cerfolio RJ, Bryant AS, Ohja B and Bartolucci AA: The maximum standardized uptake values on positron emission tomography of a non-small cell lung cancer predict stage, recurrence, and survival. J Thorac Cardiovasc Surg 130: 151-159, 2005.
12. Sasaki R, Komaki R, Macapinlac H, Erasmus J, Allen P, Forster K, Putnam JB, Herbst RS, Moran CA, Podoloff DA, Roth JA and Cox JD: [18F]fluorodeoxyglucose uptake by positron emission tomography predicts outcome of non-small-cell lung cancer. J Clin Oncol 23: 1136-1143, 2005.
13. Cerfolio RJ and Bryant AS: Maximum standardized uptake values on positron emission tomography of esophageal cancer predicts stage, tumor biology, and survival. Ann Thorac Surg 82: 391-395, 2006.
14. Cheze-Le Rest C, Metges JP, Teyton P, Jestin-Le Tallec V, Lozac'h P, Volant A and Visvikis D: Prognostic value of initial fluorodeoxyglucose-PET in esophageal cancer: a prospective study. Nucl Med Commun 29: 628-635, 2008.
15. Sobin LH and Wittekind C (eds.): TMN classification of malignant tumors. 6th edition. John Wiley & Sons, New York, 2002.
16. Schisterman EF, Perkins NJ, Liu A and Bondell H: Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. Epidemiology 16: 73-81, 2005.
17. Fujita H: Present status of esophageal cancer and its treatment in Japan. Ann Thorac Cardiovasc Surg 10: 135-139, 2004.
18. Hatt M, Visvikis D, Albarghach NM, Tixier F, Pradier O and Cheze-le Rest C: Prognostic value of 18F-FDG PET image-based parameters in oesophageal cancer and impact of tumour delineation methodology. Eur J Nucl Med Mol Imaging 38: 1191-1202, 2011.
19. van Westreenen HL, Plukker JT, Cobben DC, Verhoogt CJ, Groen H and Jager PL: Prognostic value of the standardized uptake value in esophageal cancer. AJR Am J Roentgenol 185: 436-440, 2005.
20. Yasuda T, Higuchi I, Yano M, Miyata H, Yamasaki M, Takiguchi S, Fujiwara Y, Hatazawa J and Doki Y: The impact of ^{18}F-fluorodeoxyglucose positron emission tomography positive lymph nodes on postoperative recurrence and survival in resectable thoracic esophageal squamous cell carcinoma. Ann Surg Oncol 19: 652-660, 2012.
21. Thiery JP: Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2: 442-454, 2002.
22. Thiery JP and Sleeman JP: Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 7: 131-142, 2006.
23. Huber MA, Kraut N and Beug H: Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol 17: 548-558, 2005.
24. Natsugoe S, Uchikado Y, Okumura H, Matsumoto M, Setoyama T, Tamotsu K, Kita Y, Sakamoto A, Owaki T, Ishigami S and Aikou T: Snail plays a key role in E-cadherin-preserved esophageal squamous cell carcinoma. Oncol Rep 17: 517-523, 2007.
25. Sasaki K, Natsugoe S, Ishigami S, Matsumoto M, Okumura H, Setoyama T, Uchikado Y, Kita Y, Tamotsu K, Sakamoto A, Owaki T and Aikou T: Significance of Twist expression and its association with E-cadherin in esophageal squamous cell carcinoma. J Exp Clin Cancer Res 28: 158, 2009.
26. Usami Y, Satake S, Nakayama F, Matsumoto M, Ohnuma K, Komori T, Semba S, Ito A and Yokozaki H: Snail-associated epithelial-mesenchymal transition promotes oesophageal squamous cell carcinoma motility and progression. J Pathol 215: 330-339, 2008.
27. Li Y, Ma J, Guo Q, Duan F, Tang F, Zheng P, Zhao Z and Lu G: Overexpression of MMP-2 and MMP-9 in esophageal squamous cell carcinoma. Dis Esophagus 22: 664-667, 2009.
28. Mori M, Mimori K, Sadanaga N, Inoue H, Tanaka Y, Mafune K, Ueo H and Barnard GF: Prognostic impact of tissue inhibitor of matrix metalloproteinase-1 in esophageal carcinoma. Int J Cancer 88: 575-578, 2000.
29. Li K, He W, Lin N, Wang X and Fan QX: Downregulation of N-cadherin expression inhibits invasiveness, arrests cell cycle and induces cell apoptosis in esophageal squamous cell carcinoma. Cancer Invest 28: 479-486, 2010.
30. Fernando RI, Castillo MD, Litzinger M, Hamilton DH and Palena C: IL-8 signaling plays a critical role in the epithelial-mesenchymal transition of human carcinoma cells. Cancer Res 71: 5296-5306, 2011.
31. Kato H, Takita J, Miyazaki T, Nakajima M, Fukai Y, Masuda N, Fukuchi M, Manda R, Ojima H, Tsukada K, Kuwano H, Oriuchi N and Endo K: Correlation of 18-F-fluorodeoxyglucose (FDG) accumulation with glucose transporter (Glut-1) expression in esophageal squamous cell carcinoma. Anticancer Res 23: 3263-3272, 2003.
32. Lee SY, Jeon HM, Ju MK, Kim CH, Yoon G, Han SI, Park HG and Kang HS: Wnt/Snail signaling regulates cytochrome C oxidase and glucose metabolism. Cancer Res 72: 3607-3617, 2012.
33. Langer R, Specht K, Becker K, Ewald P, Bekesch M, Sarbia M, Busch R, Feith M, Stein HJ, Siewert JR and Hofler H: Association of pretherapeutic expression of chemotherapy-related genes with response to neoadjuvant chemotherapy in Barrett carcinoma. Clin Cancer Res 11: 7462-7469, 2005.