

Correlation of the Standardized Uptake Value in FDG-PET with the Expression Level of Cell-Cycle–Related Molecular Biomarkers in Resected Non-Small Cell Lung Cancers

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Purpose: The aim of this study was to clarify the relationship between the maximum standardized uptake value (maxSUV) and the expression levels of cell-cycle–related molecular biomarkers.

Patients and Methods: Thirty consecutive patients with non-small cell lung cancer (NSCLC) were enrolled in the study. Histologically, the tumors included 23 adenocarcinomas and 7 squamous cell carcinomas. Protein expressions of Ki-67, proliferating cell nuclear antigen (PCNA), and p53 were examined by immunohistochemistry.

Results: The maxSUV was higher in poorly differentiated NSCLCs than in well-differentiated and moderately differentiated tumors ($p < 0.05$). The Ki-67 labeling index was higher in squamous cell carcinomas than in adenocarcinomas ($p < 0.05$), and also in poorly differentiated tumors than in well-differentiated and moderately differentiated tumors ($p < 0.01$). A positive correlation was found between the maxSUV and Ki-67 expression level ($r = 0.687$, $p < 0.001$). No correlation was found between maxSUV and PCNA expression ($r = 0.214$, $p = 0.248$) or between maxSUV and p53 expression ($r = 0.357$, $p = 0.09$). Among the molecular biomarkers, an association was found between the expression levels of Ki-67 and PCNA ($r = 0.515$, $p < 0.01$).

Conclusions: Immunohistochemical staining with Ki-67 in NSCLC correlates with maxSUV. Measurement of the maxSUV by PET is a simple and noninvasive method to determine the biological cancer cell proliferation potential. (*Ann Thorac Cardiovasc Surg* 2009; 15: 304–310)

Key words: fluorodeoxyglucose, Ki-67, non-small cell lung cancer, positron emission tomography, p53

Introduction

The maximum standardized uptake value (maxSUV) in fluorodeoxyglucose positron emission tomography (FDG-PET) reflects the glucose metabolic activity of a tumor. FDG accumulation seems to be associated with several factors, including the expression levels of membrane glucose transporters such as GLUT-1 and GLUT-3, the grade of tumor cell differentiation, and clinicopathological prognostic factors, including disease stage and tumor-doubling time.^{1–3} Moreover, a close association between higher SUV and reduced survival in patients with lung cancer has been demonstrated in many studies.^{4–7} The

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response to chemotherapy can be assessed by changes of SUV in pre- and posttreatment in non-small cell and small cell lung cancers,^{8,9} and in *in vitro* studies, gefitinib-sensitive cell lines have shown a dramatic decrease in FDG uptake after exposure to gefitinib, suggesting that the clinical response to it can also be evaluated by changes in SUV.¹⁰

All of the above PET studies suggest that SUV is an indicator of the potential for cancer cell proliferation, and the association of a worse prognosis in lung cancer with increased expression of cell proliferation biomarkers has been reported frequently.^{11,12} However, only a few studies have made a direct comparison of SUV with expression levels of cell proliferation biomarkers in non-small cell lung cancers (NSCLCs). Therefore the aim of this study was to clarify the relationship between the maxSUV in preoperative FDG-PET/CT and the labeling indexes (percentage of positive tumor cells) of representative cell-cycle-related molecular biomarkers, Ki-67, proliferating cell nuclear antigen (PCNA), and p53 in resected NSCLC specimens.

Patients and Methods

Patients

The study was approved by the Ethics Committee of Atami Hospital, International University of Health and Welfare. Written informed consent for the performance of FDG-PET/CT and immunohistochemical analysis of the resected tumor was obtained from all patients. FDG-PET/CT was performed within 2 weeks before surgery, and the maxSUVs in primary NSCLCs were measured and recorded by an experienced nuclear medicine radiologist. The patients included 9 men and 21 women 48 to 86 years old (mean \pm standard deviation (SD): 72.0 ± 7.7 years old). Histologically, the tumors included 23 adenocarcinomas and 7 squamous cell carcinomas; the differentiation grade was well differentiated in 10, moderately differentiated in 11, and poorly differentiated in 9. The pathological tumor node metastases (TNM) stages determined according to the International Lung Cancer Staging System¹³ were stage I in 18 cases, stage II in 6, stage III in 5, and stage IV in 1.

Imaging by FDG-PET/CT

FDG-PET/CT was performed with an integrated PET/CT scanner (GE Discovery ST, GE Healthcare, Milwaukee, WI). After the patients fasted for 4 h, they were given 250 to 300 MBq of FDG intravenously, and PET was

performed 1 h later. Patients with blood glucose concentrations exceeding 200 mg/dl at the time of examination were excluded from the study. The scanning time for positron emission was 3.5 min per image slice, and slices were obtained from the top of the skull to the midhigh. The maxSUV of each primary lung cancer lesion was calculated automatically after delineation of the region of interest on attenuation-corrected FDG-PET/CT images.

Immunohistochemical analysis

The resected lung cancer tissues were fixed in 10% formaldehyde solution followed by embedding in paraffin. Immunohistochemical staining was performed using commercially available primary mouse monoclonal antibodies for the specific antigens: MIB-1 (DakoCytomation, Copenhagen, Denmark) to Ki-67 antigen, PC10 (Dako) to PCNA, and DO-7 (Dako) to p53. All the antibodies were prediluted by the manufacturers.

The EnvisionTM dual link staining kit[®] (Dako) was used to detect the presence of specific antigens according to the manufacturer's instructions. Paraffin-embedded sections (5 μ m) were dewaxed and rehydrated. After endogenous peroxidase activity was quenched by incubating the specimen for 10 min with 0.3% hydrogen peroxidase containing sodium azide and levamisole, slides were heated at 120°C for 20 min in antigen-activation solution[®] (pH 9.0) (Nichirei Bioscience, Tokyo, Japan) to expose the protein antigens. The specimen was then incubated with diluted mouse primary antibody, followed by incubation with peroxidase-labeled polymer conjugated to goat anti-mouse immunoglobulin for 30 min. Staining was completed by a 10-min incubation with 3,3'-diaminobenzidine chromogen substrate, which resulted in a brown precipitate at the antigen site, followed by counterstaining with hematoxylin.

After staining, about 600 cancer cells (200 cells in 3 different fields) were counted randomly on each slide under light microscopy by a pathologist who was blinded to the PET results. The average percentage of positive cells was calculated as the labeling index for each biomarker.

Statistical analysis

The maxSUV and labeling index of each biomarker in subgroups based on several clinicopathological categories were compared by a nonparametric Mann-Whitney U test. The nonparametric Spearman rank-order correlation coefficient (r) was used to evaluate associations between maxSUV and the expression levels of the biomarkers and associations among the biomarkers. A p value <0.05 was considered significant.

Table 1. Summary of clinical background and data

Case	Age	Gender	Histology	Differentiation	TNM stage	maxSUV	Ki-67 (%)	PCNA (%)	p53 (%)
1	63	F	Sq	WD	IIA	3.9	67.8 ± 1.5	80.0 ± 3.5	94.0 ± 0.4
2	65	F	Ad	WD	IA	3.6	2.7 ± 1.3	7.0 ± 0.4	0
3	76	F	Ad	WD	IIIA	4.3	3.7 ± 2.4	1.9 ± 0.8	0
4	59	F	Ad	WD	IB	2.3	4.5 ± 1.8	3.8 ± 3.0	0
5	74	F	Ad	MD	IA	9.5	25.3 ± 14.5	14.2 ± 8.0	34.7 ± 6.4
6	48	M	Ad	PD	IIB	12.6	36.4 ± 3.5	60.0 ± 10.7	58.6 ± 8.7
7	80	F	Sq	MD	IV	8.9	18.9 ± 14.3	35.3 ± 11.6	9.6 ± 4.9
8	68	F	Ad	MD	IA	0	0	11.9 ± 3.2	1.1 ± 0.5
9	74	F	Sq	WD	IIB	7.4	25.0 ± 9.6	21.8 ± 4.5	80.5 ± 4.1
10	80	M	Sq	WD	IA	11.1	99.7 ± 0.6	98.6 ± 0.7	90.1 ± 5.1
11	77	F	Ad	MD	IA	6	16.8 ± 2.7	48.7 ± 9.2	0.5 ± 0.2
12	66	M	Ad	PD	IA	2	14.5 ± 5.3	15.2 ± 5.1	15.2 ± 1.3
13	65	F	Ad	PD	IA	8.8	38.7 ± 8.1	23.6 ± 5.0	30.8 ± 3.5
14	71	F	Ad	PD	IIIA	4.8	79.7 ± 5.9	51.6 ± 7.0	0
15	73	M	Ad	MD	IIB	9.4	24.0 ± 9.2	15.2 ± 2.8	99.7 ± 0.6
16	79	F	Ad	PD	IIB	9.3	17.8 ± 3.3	20.6 ± 1.2	0
17	77	F	Ad	MD	IIB	4.2	13.0 ± 1.4	9.5 ± 1.3	34.8 ± 4.2
18	74	F	Sq	MD	IA	0	21.1 ± 1.7	22.4 ± 5.3	0
19	75	F	Ad	WD	IA	2.8	2.1 ± 0.6	1.6 ± 0.7	0
20	69	F	Ad	MD	IA	3.6	4.5 ± 4.5	46.1 ± 0.7	0
21	77	F	Ad	PD	IA	7.3	25.9 ± 4.2	59.3 ± 5.4	0
22	86	F	Ad	MD	IB	7.1	75.8 ± 4.8	90.9 ± 1.5	97.0 ± 1.2
23	68	F	Ad	PD	IIIA	8.1	62.2 ± 3.3	46.0 ± 7.5	0
24	69	M	Sq	PD	IB	13.6	89.7 ± 5.1	95.8 ± 1.5	0
25	70	M	Ad	WD	IB	1.8	4.6 ± 1.8	97.2 ± 1.2	0
26	80	M	Ad	MD	IIIB	8.1	26.9 ± 7.3	96.2 ± 1.6	0
27	76	M	Sq	PD	IA	6.6	39.9 ± 7.1	91.9 ± 1.3	0
28	73	F	Ad	WD	IA	2.7	9.1 ± 1.4	94.7 ± 2.4	4.0 ± 1.2
29	82	M	Ad	WD	IA	3.6	9.6 ± 3.7	94.5 ± 3.1	0
30	66	F	Ad	MD	IIIB	3.6	9.5 ± 7.4	94.5 ± 2.3	17.1 ± 7.9

TNM, tumor node metastases; maxSUV, maximum standardized uptake value; PCNA, proliferating cell nuclear antigen; F, female; M, male; Sq, squamous cell carcinoma; Ad, adenocarcinoma; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated.

Results

Clinicopathological profiles, maxSUV in PET, and the labeling indexes of the biomarkers in the 30 patients in the study are shown in Table 1. The maxSUV (mean ± SD) of the primary lung cancers was 5.9 ± 3.6 (range: 0 to 13.6), and 25 of the 30 NSCLCs (83%) had a maxSUV > 2.5. Representative findings from FDG-PET/CT and immunohistochemical staining of the resected tumors are shown in Fig. 1.

The patients were classified into two subgroups in three categories: histological types (adenocarcinoma vs. squamous cell carcinoma); differentiation grade (poorly vs. moderately/well differentiated); and pathological stage (IA vs. IB to IV). The maxSUV and expression levels of molecular biomarkers in each subgroup and the differences between the subgroups are shown in Table 2. Poorly differentiated NSCLCs had a higher maxSUV

compared to well-differentiated and moderately differentiated tumors ($p < 0.05$). The Ki-67 labeling index was higher in squamous cell carcinomas than in adenocarcinomas ($p < 0.05$), and it was also higher in poorly differentiated tumors ($p < 0.01$). The expression levels of PCNA and p53 did not differ significantly for any of the pairs of subgroups.

A scatter plot of maxSUV and expression levels of Ki-67 (Fig. 2) showed a positive correlation between maxSUV and Ki-67 ($r = 0.687$, $p < 0.001$). In contrast, there was no significant correlation between maxSUV and PCNA ($r = 0.214$, $p = 0.248$) or between maxSUV and p53 ($r = 0.357$, $p = 0.09$). The expression levels of Ki-67 and PCNA showed a significant correlation ($r = 0.515$, $p < 0.01$) (Fig. 3), but PCNA and p53 ($r = 0.039$, $p = 0.836$) and Ki-67 and p53 ($r = 0.306$, $p = 0.099$) were not significantly associated.

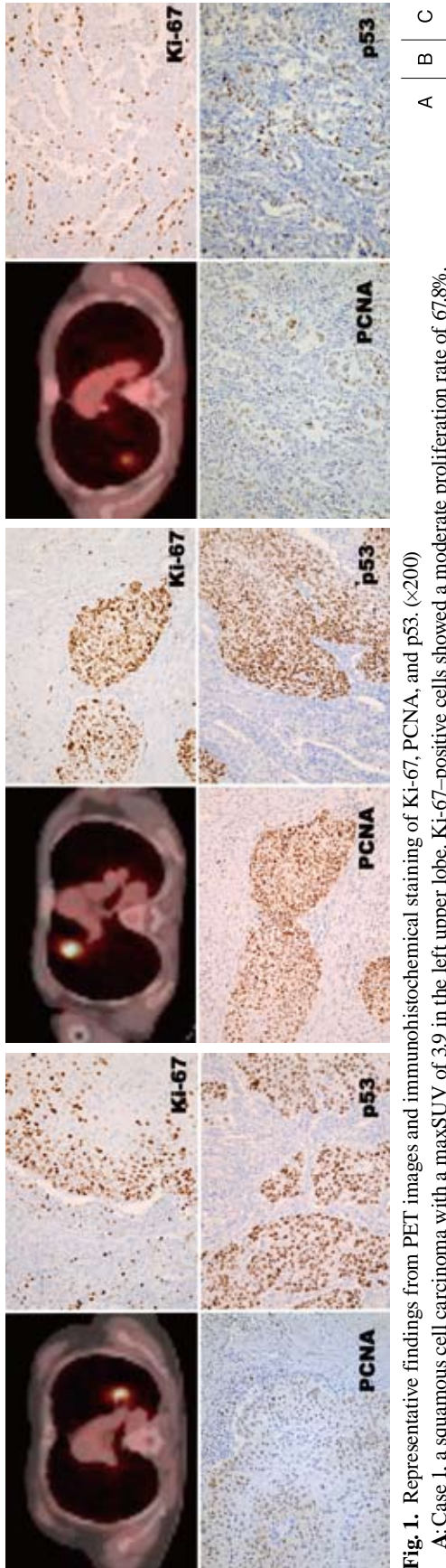


Fig. 1. Representative findings from PET images and immunohistochemical staining of Ki-67, PCNA, and p53. ($\times 200$)

A: Case 1, a squamous cell carcinoma with a maxSUV of 3.9 in the left upper lobe. Ki-67-positive cells showed a moderate proliferation rate of 67.8%.

B: Case 10, a squamous cell carcinoma with a maxSUV of 11.1 in the right upper lobe. The labeling indexes of all three biomarkers were higher than 90%.

C: Case 12, an adenocarcinoma with a maxSUV of 2.0 in the right upper lobe. The labeling indexes of all three biomarkers were about 15%.

PCNA, proliferating cell nuclear antigen.

Table 2. MaxSUVs and labeling indexes (mean \pm SD) of cell-cycle-related molecular biomarkers in NSCLCs

	Histology			Differentiation grade			Pathological stage		
	Ad (n = 23)	Sq (n = 7)	p	WD-MD (n = 21)	PD (n = 9)	p	pIA (n = 14)	pIB-IV (n = 16)	p
maxSUV	5.457 \pm 3.206	7.357 \pm 4.510	0.2386	4.948 \pm 3.167	8.122 \pm 3.595	0.0462*	4.829 \pm 3.457	6.837 \pm 3.483	0.1090
Ki-67	22.06 \pm 22.90	51.73 \pm 33.84	0.0152*	22.12 \pm 26.62	44.98 \pm 26.63	0.0093*	22.14 \pm 25.76	34.97 \pm 29.76	0.2048
PCNA	43.66 \pm 35.86	63.69 \pm 35.54	0.1479	46.95 \pm 39.42	51.56 \pm 29.17	0.5263	44.98 \pm 36.63	51.27 \pm 36.77	0.6776
p53	17.11 \pm 30.12	39.17 \pm 46.16	0.4166	26.81 \pm 39.05	11.62 \pm 20.61	0.2360	12.60 \pm 25.26	30.71 \pm 40.50	0.4239

Ad, adenocarcinoma; Sq, squamous cell carcinoma; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; maxSUV, maximum standardized uptake value; PCNA, proliferating cell nuclear antigen; *, statistically significant by a Mann-Whitney U test.

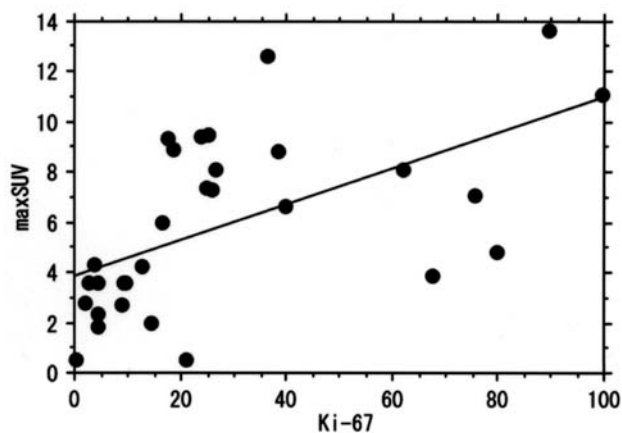


Fig. 2. Scatter plot of maxSUVs and labeling indexes of Ki-67 (%). A positive correlation was found between these values ($r = 0.687$, $p < 0.001$). maxSUV, maximum standardized uptake value.

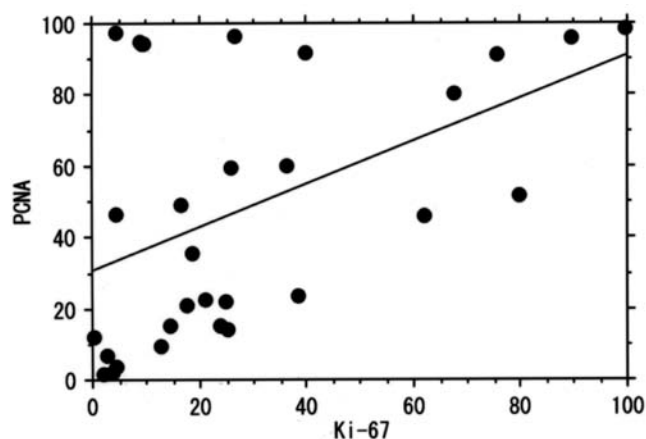


Fig. 3. Scatter plot of labeling indexes of Ki-67 (%) and PCNA (%). A positive correlation was found between these values ($r = 0.515$, $p < 0.01$). PCNA, proliferating cell nuclear antigen.

Discussion

The SUV measured in FDG-PET is a semiquantitative value that indicates the degree of glucose uptake in a lesion. MaxSUV of the primary lung nodules has been reported to be helpful in distinguishing malignant lesions from benign lesions because SUV is relatively high in malignant tumors. An early report suggested that most lung nodules with maxSUV of at least 2.5 proving to be malignant.¹⁴ However, a more recent study of a larger number of patients found a 24% chance of cancer even when a suspicious nodule had a maxSUV of less than 2.5.¹⁵ Therefore SUV may vary widely in lung cancer, and in the current study, 17% of NSCLCs had a maxSUV less than 2.5. We speculated that a tumor with increased maxSUV might be more aggressive and have a high proliferation potential, resulting in reduced survival.¹⁶ We examined this hypothesis by studying the correlation between maxSUV and the expression level of three representative cell-cycle-related molecular biomarkers, Ki-67, PCNA, and p53, because overexpression of these markers is known to worsen prognosis in NSCLC.^{11,17,18}

The Ki-67 antigen¹⁹ is detectable within the cell nucleus during all active phases of the cell cycle (G_1 , S, G_2 , and M), but it is not detectable in resting cells in G_0 . PCNA is also expressed in the nucleus during the deoxyribonucleic acid (DNA) synthesis phase in late G_1 to S, during which it interacts with DNA polymerase and RF-C protein at primer-template junctions. The p53 protein is a transcription factor that regulates the cell cycle,

and thus the p53 gene is considered to be a tumor suppressor gene. The p53 protein activates DNA repair proteins and arrests the cell cycle at the G_1/S regulation checkpoint upon recognition of DNA damage. An altered p53 protein produced by a mutated gene lacks the normal function of p53 and can accumulate in the tumor cell nucleus because of its longer half-life compared to wild-type p53.^{20,21} The Ki-67²²⁻²⁴ or PCNA^{25,26} labeling index has been reported to correlate with SUV in lung cancer, but this correlation has not always been found.²⁷ The altered expression of tumor suppressor genes, such as Rb, p16, p27, and p53, also seems to be correlated with increased SUV in lung cancer,²⁸ but the lack of a significant correlation between p53 expression and FDG-uptake has also been reported.²⁴ Thus previous studies have yielded conflicting results on the relationships of biomarkers with SUV.

In the current study, the labeling index of Ki-67 alone correlated with maxSUV in 30 cases of NSCLC. Further, expression levels of Ki-67 correlated with those of PCNA. The staining patterns for Ki-67 and PCNA were similar: some positive cells were present in all examined specimens, and the labeling index showed a wide distribution. In contrast, the staining of p53 was characterized by an “all or nothing” pattern, probably because only the mutant p53 protein can be detected immunohistochemically: we did not find a single p53-positive cancer cell in 15 of the 30 specimens (50%) in this study. Since both Ki-67 and PCNA are present in proliferating cells, we had expected similar results for these molecules, but a significant correlation with maxSUV was obtained for

only Ki-67. The prognostic significance of Ki-67 and PCNA has also been shown to differ in several other cancers.^{29,30} Because PCNA has a longer half-life than Ki-67, PCNA may remain in cells that are already in the resting G-phase,³¹ and this may make PCNA less specific. However, our results require confirmation in a larger number of patients.

Determination of the status of cancer cell invasiveness by the PET measurement of maxSUV is of importance. Because of widespread chest CT screening, small-size lung cancers are now found more frequently,³² and it is well known that lymph node metastases can be found histologically in about 20% of patients with NSCLCs of less than 2 cm.³³ Our results also show that the elevated uptake of glucose in NSCLCs, as assessed by maxSUV in PET, is correlated with an expression of a cell-cycle-related molecular biomarker, Ki-67. Measuring SUV is a simple and noninvasive method to determine the cancer cell proliferation potential, which reflects the malignant grade of the tumor, and the determination of maxSUV in a primary lesion may be useful for the selection of patients indicated for organ-sparing limited surgery or radiotherapy for small-sized NSCLCs.

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