^{18}F-FDG uptake on PET is a predictive marker of thymidylate synthase expression in patients with thoracic neoplasms

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Abstract. The aim of this study was to investigate the relationship between the expression levels of thymidylate synthase (TS) and 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) uptake on positron emission tomography (PET) in various thoracic neoplasms. In total, 392 patients [non-small cell lung cancer (NSCLC) (n=140), malignant pleural mesothelioma (MPM) (n=21), pulmonary metastatic tumors (PMT) (n=148), thymic epithelial tumors (n=49) and pulmonary neuroendocrine (NE) tumor (n=34)] who underwent ^{18}F-FDG PET before treatment were included in this study. Tumor sections were stained using immunohistochemistry for determination of TS, orotate phosphoribosyltransferase (OPRT), dihydropyrimidine dehydrogenase (DPD), vascular endothelial growth factor (VEGF), microvessel density (MVD), CD34 and p53. The expression of TS in thoracic neoplasms had a positivity of 58% (230/392), and the positive rates of TS expression in NSCLC, PMT, thymic epithelial tumor, NE tumor and MPM samples were 56, 57, 57, 85 and 47%, respectively. The positivity of TS expression was significantly higher in NE tumors compared to that in other thoracic tumors. A statistically significant correlation between TS expression and ^{18}F-FDG uptake was observed in thoracic neoplasms, in particular primary lung adenocarcinomas, high-grade NE tumors, thymomas and MPMs. Moreover, TS expression was closely associated with angiogenesis, DPD, OPRT and p53. Our results indicated that SUV$_{max}$ by ^{18}F-FDG uptake may be an alternative biomarker for predicting TS expression in patients with primary lung adenocarcinoma, high-grade NE tumor, thymoma and MPM.

Introduction

Thymidylate synthase (TS) is an enzyme that plays an important role in the DNA synthesis and catalyzes the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) (1,2). TS is also a target enzyme of 5-fluorouracil (5-FU), which is an anticancer chemotherapeutic agent for various human cancers (3). The anticancer activity of 5-FU has been described to be closely associated with the intratumoral expression of TS, orotate phosphoribosyltransferase (OPRT) and dihydropyrimidine dehydrogenase (DPD) (4). TS expression has been described to be significantly correlated with proliferative activity and poor prognosis in patients with various thoracic neoplasms such as non-small cell lung cancer (NSCLC) (5,6) and thymic epithelial tumors (7). Moreover, TS expression had been described to be significantly associated with chemotherapeutic outcome in patients with NSCLC, malignant pleural mesothelioma (MPM) and pulmonary metastatic tumor (PMT) from colorectal cancer (8-12). These studies suggest that the overexpression of TS is closely related to aggressive features and chemotherapeutic response of various thoracic neoplasms.

S-1 is an oral anticancer agent comprised of tegafur (FT), 5-chloro-2, 4-dihydroxypyridine (CDHP), and potassium oxonate (Oxo), in a molar ratio of 1:0.4:1 (13). S-1 is a potent inhibitor of DPD-inhibitory fluoropyrimidine (DIF), and is effective against patients with lung, colon and gastric cancers (4,14). Pemetrexed inhibits multiple enzymes in the folate metabolic pathway, and TS is the main target (15). *In vitro* study using NSCLC cell lines demonstrated that high expression level of *TS* gene at baseline conferred resistance to pemetrexed and TS levels were correlated with pemetrexed efficacy in a variety of solid tumors (16-18). Moreover, recent clinical studies have described that a high expression level of TS could be a possible biomarker for predicting poor outcome after TS-inhibitor treatment such as S-1 or pemetrexed in

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NSCLC or MPM (8-11). The results of these studies indicated that TS expression could be a chemoresistance protein for TS targeting therapy. However, as it is difficult to obtain an adequate specimen for immunohistochemical analysis in patients with advanced thoracic neoplasms, in selected patients, therefore, the expression of TS protein is evaluated by immunohistochemical staining.

Recently, the usefulness of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) for the diagnosis of thoracic neoplasms has been investigated (19-25). The previous studies demonstrated that the primary tumor standardized uptake value (SUV) measurement on ^{18}F-FDG PET is a useful marker for predicting outcome after treatment in patients with thoracic neoplasms (19-25). Even if an adequate specimen is not obtained, we can clearly image ^{18}F-FDG uptake within the primary tumor. The amount of ^{18}F-FDG uptake within tumor cells is determined by the glucose metabolism, hypoxia and angiogenesis ^{18}F-FDG uptake is strongly associated with the expression of glucose transporter 1 (Glut1) (24). Glut1 is thought to be a possible intrinsic marker of hypoxia, and the expression of Glut1 has been found to be regulated by hypoxia in hypoxia-inducible factor-1α (HIF-1α). Atkin *et al* (25) have documented that the direct correlation between TS expression and HIF-1α expression was recognized in primary rectal cancers, and the microenviromental factor such as acidosis or alternations in the availability of glucose and other enzymatic substrates, are more active in human cancers, thereby affecting the level of TS or HIF-1α expression. Recent immunohistochemical data demonstrated that TS expression was significantly correlated with Glut1, HIF-1α and angiogenesis in patients with primary lung cancer (6). However, it remains unclear whether the patients with various thoracic neoplasms have a significant relationship between ^{18}F-FDG uptake on PET and TS protein expression.

Based on the above background, we investigated the relationship between ^{18}F-FDG uptake on PET and TS expression in patients with various thoracic neoplasms. Moreover, correlation of TS expression was determined with OPRT, DPD, vascular endothelial growth factor (VEGF), microvessel density (MVD), CD34 and p53.

Materials and methods

Patients. Between April, 2003 and May, 2009, we analyzed 148 consecutive patients with PMT treated by lung resection for pulmonary metastasis from extrathoracic malignancies, 21 consecutive patients, 34 consecutive patients with pulmonary neuroendocrine (NE) tumors treated by curative resection, and 49 consecutive patients with thymic epithelial tumors who underwent ^{18}F-FDG PET at Shizuoka Cancer Center (Shizuoka, Japan). In 148 patients with PMT [adenocarcinoma (AC) with 106, squamous cell carcinoma (SQC) with 15, sarcoma with 20 and other with 8], the primary site was colon in 80 patients, breast in 9, head and neck in 14, genital system in 12, esophagus in 3, gastrointestinal tract in 7, soft tissue and bone in 20 and other sites in 3. In 21 patients with MPM, 16 patients had a histology of epithelial type, 2 biphasic type, 1 sarcomatous type, and 2 unspecific type. In 34 patients with pulmonary NE tumors, the pathological diagnoses were:

typical carcinoid (n=5), atypical carcinoid (n=1), small-cell lung carcinoma (SCLC) (n=12) and large cell neuroendocrine carcinoma (LCNEC) (n=16). High-grade NE tumor was SCLC and LCNEC. In 49 patients with thymic epithelial tumors, there was 38 patients with thymoma and 11 patients with thymic carcinoma.

NSCLC patients were consecutively assigned to the study between December, 2002 and March, 2004, and ^{18}F-FDG PET was performed as part of the preoperative work-up. These patients underwent surgical management, and the primary lesions were resected. Finally, 140 patients with NSCLC (95 with AC, 43 with SQC and 2 with large cell carcinoma) were evaluated.

Of the total 392 patients, 231 patients were male and 161 female. The age of the patients ranged from 16 to 89 years, and the median age was 62 years. None of the patients had insulin-dependent diabetes, and the serum glucose levels in all patients just before ^{18}F-FDG PET study was less than 120 mg/dl. The authors' approach to the evaluation and resection of these tumors has been described previously (6,7,12,23,24,26-28). The study protocol was approved by the Institutional Review Board.

Immunohistochemical staining. Immunohistochemical staining was performed according to the procedure described in the previous studies (7,24). The following antibodies were used: a rabbit polyclonal antibody against TS (clone RTSSA, 1:1,600 dilution; Taiho Pharmaceutical, Co., Ltd., Saitama, Japan); a rabbit polyclonal antibody against OPRT (1:1,200 dilution; Taiho Pharmaceutical, Co., Ltd.); a rabbit polyclonal antibody against DPD (clone RDPDPA, 1:500 dilution; Taiho Pharmaceutical, Co., Ltd.); a monoclonal antibody against VEGF (1:200 dilution; Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan); a mouse monoclonal antibody against CD34 (1:800 dilution; Nichirei, Tokyo, Japan); a mouse monoclonal antibody against p53 (D07, 1:50 dilution; Dako). Antibodies against TS, OPRT and DPD were kindly donated by Taiho Pharmaceutical, Co., Ltd. (Tokyo, Japan).

The expression of TS, OPRT and DPD was considered if nuclear or cytoplasmic staining was present. For TS, OPRT and DPD, a semi-quantitative scoring method was used: 1, <10%; 2, 10-25%; 3, 25-50%; 4, 51-75% and 5, >75% of cells stained positively. The tumors in which stained tumor cells made up more than 25% of the tumor were graded as positive.

The expression of VEGF was quantitatively assessed according to the percentage of immunoreactive cells in a total of 1,000 neoplastic cells. The number of CD34-positive vessels was counted in four selected hot spots in a 0.26 mm^2 field area. MVD was defined as the mean count of microvessels per 0.26 mm^2 field area.

For p53, microscopic examination for the nuclear reaction product was performed and scored. According to a previous study (24), p53 expression in more than 10% of tumor cells was defined as high expression. Sections were assessed using a light microscope in a blinded fashion by at least two of the authors.

^{18}F-FDG PET imaging. Patients fasted for at least 4 h before ^{18}F-FDG PET examination. Patients received an intravenous injection of 200-250 MBq of ^{18}F-FDG and then rested for

approximately 1 h before undergoing imaging (24). Image acquisition was performed using an Advance NXi PET scanner and Discovery PET-CT scanner (GE Medical Systems, Milwaukee, WI, USA). Two-dimensional emission scanning was performed from the groin to the top of the skull. PET/CT image was independently reviewed by two experienced physicians. Acquired data were reconstructed by iterative ordered subset expectation maximization. To evaluate ^{18}F-FDG accumulation, the tumor was first examined visually, and then the peak standardized uptake value (SUV) of the entire tumor was determined. SUV_{max} was defined as the peak SUV value on one pixel with the highest counts within the region of interest (ROI). The ROI, measuring 3 cm in diameter, was set at the mediastinum at the level of the aortic arch and the mean SUV of the mediastinum was calculated.

Statistical analysis. Probability values of <0.05 indicated a statistically significant difference. Fisher's exact test was used to examine the association of two categorical variables. Correlation of different variables was analyzed using the nonparametric Spearman's rank test. Statistical analysis was performed using JMP 8 (SAS Institute Inc., Cary, NC, USA) for Windows.

Results

Immunohistochemical staining and SUV_{max} by ^{18}F-FDG uptake. Each protein revealed a profile pattern of the unique expression. The immunohistochemical staining was evaluated for the 392 thoracic tumor lesions. The mean scoring (mean ± SD) of TS, OPRT and DPD was 2.54±1.00, 2.53±1.15 and 2.37±1.21, respectively. A positive expression of TS, OPRT and DPD was recognized in 58 (230/392), 46 (179/392) and 55% (217/392), respectively. The staining pattern of VEGF was uniformly localized in the cytoplasm and/or membrane of neoplastic tissue. The median rate of VEGF positivity was 25% (range, 1-88%), and the value of 25% was chosen as a cutoff point. Positive expression was recognized in 53% of cases (208/392). The median number of CD34-positive vessels was 24 (2-68), and the value of cutoff point was 24. Positive expression of CD34 was seen in 50% of cases (196/392). Positive expression of p53 was recognized in 46% of cases (180/392).

The SUV_{max} of the primary tumors in 392 patients ranged from 0.8 to 31.9 (median, 5.2). A median value of 5.2 was used as the cutoff SUV in the following analyses, and the SUV_{max} in more than 5.2 was defined as positive expression. Positive expression of SUV_{max} was seen in 50% of cases (197/392). Fig. 1 is representative imaging of TS expression and ^{18}F-FDG PET. Fig. 2 shows the rate of positive expression of these different biomarkers according to disease types.

Relationship between TS expression and different variables. Table I shows a comparison of the different variables according to TS expression. A positive TS expression was significantly correlated with male, SUV_{max} and the expression of OPTT, DPD, VEGF, CD34 and p53. In the analysis according to primary disease types, the positive rate of TS expression in NE tumor (n=34) was significantly higher than that in NSCLC (n=140; P=0.001), PMT (n=148; P=0.001), thymic epithelial
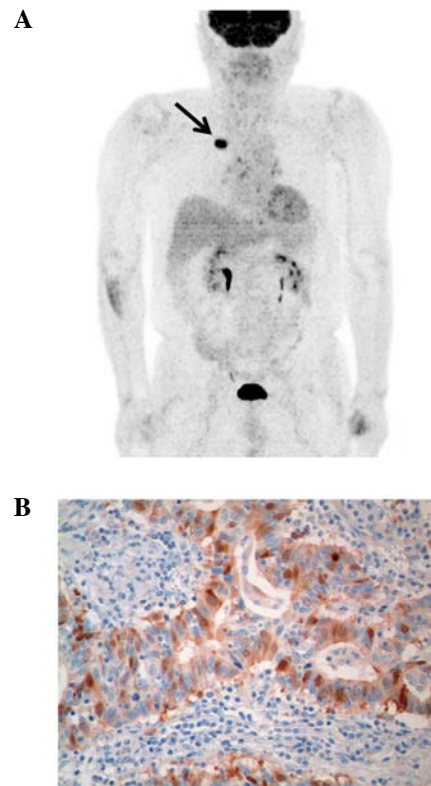

Figure 1. ^{18}F-FDG PET in a 72-year-old male with pulmonary adenocarcinoma and immunohistochemical staining of TS expression. (A) ^{18}F-FDG PET shows SUV_{max} of 8.7, and (B) TS is stained in nuclei with scoring of 3. TS, thymidylate synthase; SUV_{max}, maximal standardized uptake value.
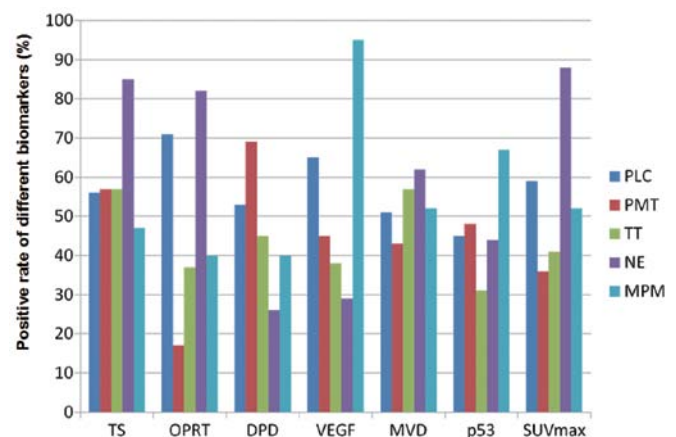

Figure 2. Positive rate according to different biomarkers (PLC, primary lung cancer; PMT, pulmonary metastatic tumor; TT, thymic epithelial tumor; NE, neuroendocrine tumor; MPM, malignant pleural mesothelioma). Positive rates of TS expression in PLC, PMT, TT, NE and MPM were 56, 57, 57, 85 and 47%, respectively. Those of OPRT, DPD, VEGF, MVD, p53 and SUV_{max} in PLC, PMT, TT, NE and MPM were 71, 17, 37, 82 and 40%, respectively, 53, 69, 45, 26 and 40%, respectively, 65, 45, 38, 29 and 95%, respectively, 51, 43, 57, 62 and 52%, respectively, 45, 48, 31, 44 and 67%, respectively, and 59, 36, 41, 88 and 52%, respectively. TS, thymidylate synthase; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase; VEGF, vascular endothelial growth factor; MVD, microvessel density determinate by CD34; SUV_{max}, maximal standardized uptake value.

tumors (n=49; P=0.008) and MPM (n=21; P=0.005). No statistically significant difference in the TS expression was observed among NSCLC, PMT, thymic epithelial tumors and

Table I. Different variables according to TS expression.

Variables	TS positive (n=230)	TS negative (n=162)	P-value
Age (≤65/>65 years)	108/122	85/77	0.305
Gender (male/female)	153/77	78/84	<0.001
Primary site (thoracic/extrathoracic)	146/84	98/64	0.597
SUV_{max} (low/high)	75/155	120/42	<0.001
DPD (low/high)	92/138	83/79	0.031
OPRT (low/high)	90/140	123/39	<0.001
VEGF (low/high)	79/151	105/57	<0.001
CD34 (low/high)	81/149	115/47	<0.001
p53 (low/high)	92/138	120/42	<0.001

TS, thymidylate synthase; SUV_{max}, maximal standardized uptake value; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase; VEGF, vascular endothelial growth factor; thoracic, primary site is thoracic lesion; extrathoracic, primary site is extrathoracic lesion.

MPM. Next, the positive rate of TS expression was compared according to histological types. Two hundred and one patients had a histology of adenocarcinoma (AC), 58 squamous cell carcinoma (SQC), 28 high-grade NE tumors, 20 sarcoma, 38 thymoma and 16 MPM with epithelial type. The positive rates of AC, SQC, high-grade NE tumors, sarcoma, thymoma and MPM with epithelial type were 51, 86, 96, 35, 47 and 47%, respectively. The positive rate of TS expression was significantly lower in patients with AC than in SQC (P<0.001) and high-grade NE tumors (P<0.001), demonstrating no significant difference (P=0.26). No significantly significant difference was recognized between AC and sarcoma, between AC and thymoma and between SQC and high-grade NE tumors. Finally, we compared the positive rate of TS expression between primary lung cancer and PMT according to histological type (AC or SQC). The positive rate of TS expression (P=0.002) in histology with AC was significantly higher in PMT (61%; n=106) than in NSCLC (39%, n=95), whereas that (P=0.021) with SQC was significantly higher in NSCLC (n=43; 93%) than in PMT (n=15; 67%). In patients with NSCLC, TS expression was significantly higher in SQC than in AC (P<0.001), however, no statistically significant difference in the TS expression was observed between AC and SQC in patients with PMT (P=0.782).

Correlation between TS expression and different variables. Table II shows the correlation between TS expression and various biomarkers according to disease types. The expression of TS was closely correlated with ^{18}F-FDG uptake, OPRT, DPD and angiogenesis (VEGF and MVD). In the analysis according to histological types, however, the relationship between TS expression and ^{18}F-FDG uptake showed a statistically significant correlation in patients with pulmonary AC, high-grade NE tumor, thymoma and MPM with epithelial type (Table III). In 58 patients with SQC, TS expression was not closely associated with these biomarkers including SUV_{max} by ^{18}F-FDG uptake. Of 11 patients with thymic carcinoma, 8 patients had a histological type of SQC, and TS expression

in thymic carcinoma was not closely correlated with ^{18}F-FDG uptake.

Discussion

This is the first study to investigate the relationship between ^{18}F-FDG uptake on PET and TS expression in patients with various thoracic tumors. The expression of TS in thoracic neoplasms had a positivity of 58% (230/392), and the positive rates of TS expression in NSCLC, PMT, thymic epithelial tumor, NE tumor and MPM were 56, 57, 57, 85 and 47%, respectively. The positivity of TS expression in NE tumors was significantly higher than that in other thoracic tumors. The analysis according to histology revealed that high-grade NE tumor or SQC had a higher positive rate of TS expression as compared with the other histological types. The relationship between TS expression and ^{18}F-FDG uptake had a statistically significant correlation in primary lung AC, high-grade NE tumor, thymoma and MPM. Our results indicated that SUV_{max} by ^{18}F-FDG uptake may be an alternative biomarker for predicting TS expression in patients with primary lung AC, high-grade NE tumor, thymoma and MPM.

High-level TS expression is related to an aggressive tumor phenotype and a poor outcome in a variety of malignant tumors (5). Several researchers have documented that TS level is generally lower in AC than in SQC (29,30). In lung cancer, TS expression has been described to be higher in NE tumor than in SQC (21). In thymic epithelial tumors, TS expression was correlated with the grade of malignancy and was closely associated with poor outcome (7). Recently, Takeda *et al* (8) reported that a low expression level of TS was associated with a better response and longer survival in advanced NSCLC treated by chemotherapeutic regimens including S-1, and TS expression was considered as predictive biomarkers of S-1 treatment. Although their study included a small sample size (n=22), 16 (73%) patients had AC, 1 patient SQC and 5 patients other histology. Their preliminary study suggests that TS expression seems to be a predictive marker for S-1 treat-

Table II. Correlation between TS expression and biomarkers according to the primary sites.

	SUV$_{max}$	OPRT	DPD	VEGF	CD34
Total (n=392)					
Spearman γ	0.464	0.460	0.134	0.384	0.419
95% CI	0.381-0.541	0.373-0.539	0.030-0.236	0.293-0.467	0.331-0.499
P-value	**<0.001**	**<0.001**	**0.009**	**<0.001**	**<0.001**
Primary lung cancer (n=140)					
Spearman γ	0.596	0.583	-0.103	0.634	0.551
95% CI	0.473-0.696	0.457-0.685	-0.269-0.068	0.520-0.726	0.419-0.659
P-value	**<0.001**	**<0.001**	**0.224**	**<0.001**	**<0.001**
Pulmonary metastatic tumors (n=148)					
Spearman γ	0.201	0.311	0.395	0.321	0.186
95% CI	0.036-0.355	0.153-0.454	0.245-0.527	0.163-0.462	0.021-0.341
P-value	0.014	0.001	<0.001	<0.001	0.023
Thymic epithelial tumors (n=49)					
Spearman γ	0.616	0.650	0.636	0.755	0.525
95% CI	0.398-0.768	0.444-0.791	0.425-0.781	0.596-0.857	0.279-0.707
P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Neuroendocrine tumors (n=34)					
Spearman γ	0.634	0.788	-0.069	0.336	0.567
95% CI	0.367-0.804	0.607-0.891	-0.406-0.285	-0.012-0.612	0.274-0.764
P-value	**<0.001**	**<0.001**	**0.697**	**0.052**	**<0.001**
Malignant pleural mesothelioma (n=21)					
Spearman γ	0.665	0.446	0.709	0.480	0.554
95% CI	0.315-0.855	0.004-0.742	0.388-0.786	0.047-0.761	0.148-0.801
P-value	**0.001**	**0.042**	**<0.001**	**0.027**	**0.009**

TS, thymidylate synthase; SUV$_{max}$, maximal standardized uptake value; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase; VEGF, vascular endothelial growth factor; CI, confidential interval.

ment in patients with AC. Sun et al (9) described the clinical significance of TS expression in 193 patients with advanced non-squamous NSCLC treated with pemetrexed-based chemotherapy, and higher response rates for pemetrexed-based chemotherapy were associated with TS-negativity, and survival in pemetrexed-based chemotherapy was significantly longer in groups with TS-negativity. Two studies have described that a low expression level of TS are predictive of improved outcome in patients with MPM treated by pemetrexed-based chemotherapy (10,11). In patients with PMT, however, it remains unknown whether TS protein expression within pulmonary metastatic tumors is associated with aggressiveness and poor outcome. The results of these studies indicated that TS expression could be a prognostic and predictive marker for outcome after chemotherapy including TS-inhibitor regimens.

Currently, paraffin-embedded specimens obtained by biopsy are the usual materials available for immunohistochemical analysis in patients with NSCLC and MPM treated by chemotherapy. But, these tumor samples are sometimes too small for the detection of molecular markers in heterogeneous tumor tissue by immunohistochemistry. In NSCLC or MPM with advanced disease, if an adequate specimen is not available for immunohistochemical staining, the biopsy may bias the immunohistochemical analysis of TS protein expression.

However, ^{18}F-FDG PET is appropriate for the detection of primary tumor lesions in patients with advanced NSCLC or MPM (19,21). In our study, SUV$_{max}$ by ^{18}F-FDG uptake was correlated with the expression level of TS in patients with primary lung AC and MPM. Our recent study documented that TS expression was significantly correlated with Glut1, HIF-1α and angiogenesis in patients with primary lung AC, but not primary lung SQC (6), although it remains unclear whether these hypoxic markers in MPM patients are closely related to the expression level of TS. Therefore, it may be reasonable that SUV$_{max}$ by ^{18}F-FDG uptake could be an alternative marker for the expression of TS in patients with primary lung AC. Further study is warranted to evaluate whether the SUV$_{max}$ could be a useful biomarker for predicting the chemoresistance to TS-inhibitor regimens (chemotherapy including S-1 or pemetrexed) for these patients.

The patients with primary lung SQC had a high uptake of ^{18}F-FDG and a high expression of TS, demonstrating no statistically significant correlation, thus SUV$_{max}$ by ^{18}F-FDG uptake was not useful for predicting the chemoresistance to TS-inhibitor treatment in patients with SQC. Since TS-inhibitor regimens are not generally administered to patients with high-grade NE tumor and thymoma, further study is warranted for evaluating whether the expression level of TS could be

Table III. Correlation between TS expression and biomarkers according to the histological types.

	SUV$_{max}$	OPRT	DPD	VEGF	CD34
Adenocarcinoma					
Total patients (n=201)					
Spearman γ	0.298	0.225	0.271	0.363	0.351
95% CI	0.161-0.421	0.008-0.355	0.133-0.398	0.232-0.481	0.218-0.469
P-value	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**
Primary lung cancer (n=95)					
Spearman γ	0.575	0.503	0.240	0.592	0.478
95% CI	0.418-0.699	0.331-0.643	0.034-0.426	0.438-0.712	0.301-0.623
P-value	**<0.001**	**<0.001**	**0.019**	**<0.001**	**<0.001**
Pulmonary metastatic tumors (n=106)					
Spearman γ	0.114	0.261	0.259	0.202	0.141
95% CI	-0.083-0.304	0.069-0.435	0.066-0.433	0.006-0.383	-0.056-0.328
P-value	0.241	**0.006**	**0.007**	**0.037**	0.147
Squamous cell carcinoma					
Total patients (n=58)					
Spearman γ	0.222	0.202	-0.104	0.256	0.115
95% CI	-0.045-0.461	-0.067-0.443	-0.360-0.165	-0.009-0.488	-0.155-0.369
P-value	0.093	0.128	0.437	0.052	0.389
Primary lung cancer (n=43)					
Spearman γ	0.285	0.150	-0.101	0.309	0.1765
95% CI	-0.025-0.546	-0.166-0.438	-0.397-0.214	0.001-0.5646	-0.139-0.460
P-value	0.063	0.335	0.520	**0.043**	0.257
Pulmonary metastatic tumors (n=15)					
Spearman γ	0.103	0.277	0.174	0.076	-0.035
95% CI	-0.445-0.595	-0.289-0.700	-0.385-0.641	-0.466-0.578	-0.549-0.498
P-value	0.714	0.316	0.534	0.786	0.900
High-grade neuroendocrine tumors (n=28)					
Spearman γ	0.395	0.729	-0.274	0.242	0.476
95% CI	0.014-0.675	0.480-0.869	-0.594-0.121	-0.154-0.573	0.113-0.726
P-value	**0.037**	**<0.001**	0.157	0.213	**0.010**
Sarcoma (n=20)					
Spearman γ	0.246	0.331	0.559	0.416	0.116
95% CI	-0.233-0.629	-0.145-0.682	0.141-0.808	-0.046-0.732	-0.356-0.542
P-value	0.295	0.154	**0.010**	0.068	0.624
Thymoma (n=38)					
Spearman γ	0.489	0.529	0.632	0.701	0.409
95% CI	0.192-0.704	0.243-0.731	0.383-0.795	0.483-0.837	0.093-0.651
P-value	**0.002**	**<0.001**	**<0.001**	**<0.001**	**0.012**
Thymic carcinoma (n=11)					
Spearman γ	-0.055	0.588	0.137	0.345	-0.224
95% CI	-0.646-0.577	-0.038-0.883	-0.519-0.692	-0.339-0.791	-0.735-0.451
P-value	0.872	0.056	0.687	0.297	0.508
Malignant pleural mesothelioma (epithelial type) (n=16)					
Spearman γ	0.614	0.475	0.729	0.423	0.496
95% CI	0.153-0.855	-0.043-0.792	0.352-0.903	-0.107-0.766	-0.015-0.802
P-value	**0.012**	0.063	**0.001**	0.102	**0.051**

TS, thymidylate synthase; SUV$_{max}$, maximal standardized uptake value; OPRT, orotate phosphoribosyltransferase; DPD, dihydropyrimidine dehydrogenase; VEGF, vascular endothelial growth factor; CI, confidential interval.

predictive of outcome after TS-targeting therapy in these populations.

In conclusion, TS is highly expressed in high-grade NE tumors. TS expression has a statistically significant correlation with SUV_{max} by ^{18}F-FDG uptake in primary lung AC, high-grade NE tumors, thymoma and MPM. Considering that TS is a possible marker for predicting chemoresistance to TS targeting therapy such as S-1 or pemetrexed, SUV_{max} by ^{18}F-FDG uptake in primary lung AC may be an alternative marker for the expression level of TS. Further study is warranted for investigating whether SUV_{max} by ^{18}F-FDG uptake could be a useful marker for predicting outcome after S-1 or pemetrexed treatment.

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