Li D, Ambrogio L, Shimamura T, Kubo S, Takahashi M, Chirieac LR, Padera RF, Shapiro GI, Baum A, Himmelsbach F, Rettig WJ, Meyerson M, Solca F, Greulich H, Wong KK (2008) BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. *Oncogene* **27:** 4702–4711

Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* **350:** 2129–2139

Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saijo Y, Hagiwara K, Morita S, Nukiwa T (2010) Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* **362:** 2380–2388

Maruyama R, Nishiwaki Y, Tamura T, Yamamoto N, Tsuboi M, Nakagawa K, Shinkai T, Negoro S, Imamura F, Eguchi K, Takeda K, Inoue A, Tomii K, Harada M, Masuda N, Jiang H, Itoh Y, Ichinose Y, Saijo N, Fukuoka M (2008) Phase III study, V-15-32, of gefitinib versus docetaxel in previously treated Japanese patients with non-small-cell lung cancer. *J Clin Oncol* **26:** 4244–4252

Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakami N, Takada M, Yoshioka H, Shibata K, Kudoh S, Shimizu E, Saito H, Toyooka S, Nakagawa K, Fukuoka M (2010) Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* **11:** 121–128

Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M (2009a) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* **361:** 947–957

Mok TS, Wu YL, Yu CJ, Zhou C, Chen YM, Zhang L, Ignacio J, Liao M, Srimuninnimit V, Boyer MJ, Chua-Tan M, Sriuranpong V, Sudoyo AW, Jin K, Johnston M, Chui W, Lee JS (2009b) Randomized, placebo-controlled, phase II study of sequential erlotinib and chemotherapy as first-line treatment for advanced non-small-cell lung cancer. *J Clin Oncol* **27:** 5080–5087

Nakai H, Fukuoka M, Furuse K, Nakao I, Yoshimori K, Ogura T, Hara N, Sakata Y, Saito H, Hasegawa K (1991) An early phase II study of CPT-11 in primary lung cancer. *Gan To Kagaku Ryoho* **18:** 607–612

Negoro S, Fukuoka M, Niitani H, Suzuki A, Nakabayashi T, Kimura M, Motomiya M, Kurita Y, Hasegawa K, Kuriyama T (1991) [A phase II study of CPT-11, a camptothecin derivative, in patients with primary lung cancer. CPT-11 Cooperative Study Group]. *Gan To Kagaku Ryoho* **18:** 1013–1019

Negoro S, Masuda N, Takada Y, Sugiura T, Kudoh S, Katakami N, Ariyoshi Y, Ohashi Y, Niitani H, Fukuoka M (2003) Randomised phase III trial of irinotecan combined with cisplatin for advanced non-small-cell lung cancer. *Br J Cancer* **88:** 335–341

Ohtsuka K, Ohnishi H, Morii T, Fujiwara M, Kishino T, Ogura W, Chiba M, Matsushima S, Goya T, Watanabe T (2010) Downregulated ABCG2 enhances sensitivity to topoisomerase I inhibitor in epidermal growth factor receptor tyrosine kinase inhibitor-resistant non-small cell lung cancer. *J Thorac Oncol* **5:** 1726–1733

Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304:** 1497–1500

Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, Varmus H (2005) Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* **2:** e73

Park K, Goto K (2006) A review of the benefit-risk profile of gefitinib in Asian patients with advanced non-small-cell lung cancer. *Curr Med Res Opin* **22:** 561–573

Rizvi NA, Kris MG, Miller VA, Krug LM, Bekele S, Dowlati A, Rowland KM, Salgia R, Aggarwal N, Gadgeel SM (2008) Activity of XL647 in clinically selected NSCLC patients (pts) enriched for the presence of EGFR mutations: results from Phase 2. *Proc Am Soc Clin Onc* **26**(Suppl): abstract 8063

Rusch V, Baselga J, Cordon-Cardo C, Orazem J, Zaman M, Hoda S, McIntosh J, Kurie J, Dmitrovsky E (1993) Differential expression of the epidermal growth factor receptor and its ligands in primary non-small cell lung cancers and adjacent benign lung. *Cancer Res* **53:** 2379–2385

Sequist LV, Besse B, Lynch TJ, Miller VA, Wong KK, Gitlitz B, Eaton K, Zacharchuk C, Freyman A, Powell C, Ananthakrishnan R, Quinn S, Soria JC (2010) Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: results of a phase II trial in patients with advanced non-small-cell lung cancer. *J Clin Oncol* **28:** 3076–3083

Shimoyama T, Koizumi F, Fukumoto H, Kiura K, Tanimoto M, Saijo N, Nishio K (2006) Effects of different combinations of gefitinib and irinotecan in lung cancer cell lines expressing wild or deletional EGFR. *Lung Cancer* **53:** 13–21

Stewart CF, Leggas M, Schuetz JD, Panetta JC, Cheshire PJ, Peterson J, Daw N, Jenkins III JJ, Gilbertson R, Germain GS, Harwood FC, Houghton PJ (2004) Gefitinib enhances the antitumor activity and oral bioavailability of irinotecan in mice. *Cancer Res* **64:** 7491–7499

Tang Z, Du R, Jiang S, Wu C, Barkauskas DS, Richey J, Molter J, Lam M, Flask C, Gerson S, Dowlati A, Liu L, Lee Z, Halmos B, Wang Y, Kern JA, Ma PC (2008) Dual MET-EGFR combinatorial inhibition against T790M-EGFR-mediated erlotinib-resistant lung cancer. *Br J Cancer* **99:** 911–922

Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K (2005) Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* **366:** 1527–1537

Yang CH, Huang CJ, Yang CS, Chu YC, Cheng AL, Whang-Peng J, Yang PC (2005) Gefitinib reverses chemotherapy resistance in gefitinib-insensitive multidrug resistant cancer cells expressing ATP-binding cassette family protein. *Cancer Res* **65:** 6943–6949

Med Oncol
DOI 10.1007/s12032-011-0069-8

ORIGINAL PAPER

Thymidylate synthase expression is closely associated with outcome in patients with pulmonary adenocarcinoma

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Received: 29 June 2011 / Accepted: 8 September 2011

Abstract The aim of this study is to elucidate the prognostic significance of thymidylate synthase (TS), orotate phosphoribosyltransferase (OPRT) and dihydropyrimidine dehydrogenase (DPD) in completely resected non-small cell lung cancer (NSCLC). One hundred and sixty patients with NSCLC were included in this study. Tumor sections were stained by immunohistochemistry for TS, OPRT, DPD, glucose transporter 1 (Glut1), hypoxia inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), microvessel density (MVD) determinated by CD34, epidermal growth factor receptor (EGFR), phosph-Akt, phosph-mammalian target of rapamycin (mTOR) and p53. TS, OPRT and DPD were positively expressed in 46, 71 and 54%, respectively. The expression of TS and OPRT was significantly higher in patients with non-adenocarcinoma (non-AC) ($n = 53$) than adenocarcinoma (AC) ($n = 107$), and DPD expression was higher in adenocarcinoma as compared with non-adenocarcinoma. A positive TS expression was an independent prognostic factor for predicting a poor outcome in patients with AC, but not in those with non-AC. In AC patients, TS expression was significantly associated with advanced stage, lymph node metastases, vascular invasion, Glut1, HIF-1α, angiogenesis, EGFR signaling pathway and p53. In patients with non-AC, TS expression was not closely correlated with outcome and these biomarkers. A positive TS expression was a powerful prognostic factor to predict a poor outcome in completely resected AC patients.

Keywords TS · OPRT · DPD · NSCLC · Adenocarcinoma · Prognosis

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Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death and has a dismal prognosis. To improve the outcome of NSCLC patients, the biomarkers that may predict the prognosis and response to the specific therapy should be established. Tumor staging and performance status have been consistently shown to be the most powerful prognostic tool for predicting the outcome of NSCLC patients [1]. However, there has been no established clinical marker, which correlates with the response to the treatment and the prognosis in patients with NSCLC.

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) [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

Published online: 24 September 2011

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 NSCLC [5]. Several researchers had documented that TS expression in patients with NSCLC is significantly higher in squamous cell carcinoma (SQC) compared with adenocarcinoma (AC), and a positive correlation is observed between TS expression and disease stage, lymph node metastasis or tumor differentiation [6, 7]. However, it is unclear whether the prognostic significance of TS expression is different according to the histology of NSCLC. Recently, TS expression had been described to be correlated with the hypoxic markers such as hypoxia inducible factor-1α (HIF-1α), glucose transporter 1 (Glut1) and vascular endothelial growth factor (VEGF) in colorectal cancer [8]. Moreover, experimental study using NSCLC cell lines had documented that the combination of oral fluorouracil S-1 and epidermal growth factor receptor (EGFR) inhibitor gefitinib showed a synergistic antitumor effect, and gefitinib induced the down-regulation of TS [9]. These reports suggest that TS expression is related to EGFR signaling pathway and hypoxic condition in cancers. However, it remains unknown whether TS expression is associated with hypoxia-related protein or EGFR signaling pathway in human neoplasm.

Nakano et al. [4] reported the prognostic role of TS, OPRT and DPD for NSCLC patients postoperatively treated by a combination of tegafur and uracil (UFT). However, it remains unknown whether OPRT and DPD expression has a prognostic role in NSCLC patients without 5-FU adjuvant therapy.

To elucidate the prognostic role of TS, OPRT and DPD, we conducted an immunohistochemical examination of these biomarkers in patients with resected NSCLC. In addition, the relationship between TS expression, and hypoxia-related protein [HIF-1α, Glut1, VEGF and microvessel density (MVD) determined by CD34], EGFR signaling pathway [EGFR, phosph-Akt and phosph-mammalian target of rapamycin (mTOR)] and cell cycle regulator (p53) were also presented.

Materials and methods

Patients

Between October 2002 and September 2004, we analyzed 173 consecutive patients with NSCLC who underwent resection by either lobectomy or pneumonectomy with mediastinal lymph node dissection at Shizuoka Cancer Center. Of these patients, 13 patients were excluded for further studies because the tissue specimens were not available. Thus, a total of 160 patients (97 men, 63 women) were eligible in the study. The study protocol was approved by the institutional review board.

The age of the patients ranged from 43 to 83 years, and the median age at the time of surgery was 67 years. The tumor specimens were histologically classified according to the criteria of the World Health Organization. Pathologic tumor-node-metastasis (TNM) stages were established using the International System for Staging Lung Cancer adopted by the American Joint Committee on Cancer and the Union Internationale Centre le Cancer [10]. Histologically, 107 patients had adenocarcinoma (AC), 48 had squamous cell carcinoma (SQC), and five had large cell carcinoma (LCC). Of the total patients, 106, 25 and 29 had stage I, II and III diseases, respectively. The patients who underwent lobectomy and pneumonectomy were 104 and two patients in stage I, respectively, 23 and two patients in stage II, respectively, and 24 and five patients in stage III, respectively. As postoperative adjuvant therapies, platinum-based chemotherapy, radiation and oral administration of tegafur (a fluorouracil derivative drug) were carried out in 2, 1 and 6 patients, respectively. The two patients who received platinum-based chemotherapy (carboplatin plus paclitaxel) had stage III, the one patient treated by radiation had stage III, and the six patients receiving oral administration of tegafur had stage I. Intraoperative therapy was not performed on any patient. The postoperative clinical course was assessed by analyzing outpatient medical records and by marking telephone inquiries. The day of surgery was considered the starting day for counting postoperative survival. The follow-up duration ranged from 7 to 102 months (median, 73 months).

Immunohistochemical staining

Immunohistochemical staining was performed according to the procedure described in the previous reports [4, 11]. The following antibodies were used: a rabbit polyclonal antibody against TS (clone RTSSA; Taiho Pharmaceutical, Saitama, Japan; 1:1,600 dilution); a rabbit polyclonal antibody against OPRT (Taiho Pharmaceutical, Saitama, Japan; 1:1,200 dilution); a rabbit polyclonal antibody against DPD (clone RDPDPA; Taiho Pharmaceutical, Saitama, Japan; 1:500 dilution); a rabbit polyclonal against GLUT1 (AB15309, Abcam, Tokyo, Japan, 1:400 dilution); a mouse monoclonal against HIF-1α (NB100-123, Novus Biologicals, Inc., Littleton, 1:50 dilution); a monoclonal antibody against VEGF (Immuno-Biological Laboratories Co.,Ltd., Japan, 1:100 dilution); a mouse monoclonal antibody against CD34 (Nichirei, Tokyo, Japan, 1:800 dilution); a mouse monoclonal antibody against EGFR (Novovastra laboratories Ltd., Newcastle, UK, 1:100

dilution); a rabbit polyclonal antibody against phosph-Akt (Abcam, Tokyo, Japan, 1:200 dilution); a rabbit monoclonal antibody against phosph-mTOR (Cell signaling, 80 dilution); a mouse monoclonal antibody against p53 (D07; DAKO, 1:50 dilution). Antibodies against TS, OPRT and DPD were kindly donated by Taiho (Tokyo, Japan). The human colon cancer cell line DLD-1/FrUrd was used as a positive control for the staining of TS. Sections of resected lung tumors to express OPRT were used as positive controls for the staining of OPRT. The human pancreatic cancer cell line MIAPaCa-2 was used as a positive control for the staining of DPD. For negative control, incubation step with the primary antibody was omitted.

The expression of TS, OPRT and DPD was considered if nuclei or cytoplasm staining was present. For TS, OPRT and DPD, a semiquantitative scoring method was used: 1 = <10%, 2 = 10–24%, 3 = 25–50%, 4 = 51–75% and 5 = >75% of cells positive. The tumors in which stained tumor cells made up more than 25% of the tumor were graded as positive.

The expression of Glut1 and EGFR was considered positive if distinct membrane staining was present. Five fields (X400) were analyzed to determine the frequency of the HIF-1α-stained nuclei. p-Akt and p-mTOR were considered positive if membranous and/or cytoplasmic staining was present. For Glut1, HIF-1α,EGFR, p-Akt and p-mTOR, a semiquantitative scoring method was used: 1 = <10%, 2 = 10–24%, 3 = 25–50%, 4 = 51–75% and 5 = >75% of cells positive. 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 X 400 field (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 previous report, p53 expression in more than 10% of tumor cells was defined as high expression. Sections were assessed using a light microscopic in a blinded fashion by at least two of the authors. To test interobserver variability, each section was reassessed by the same investigators after completion of the first assessment. The time interval between the first and second assessments was at least 4 weeks. Intraobserver variability was also determined by comparing the values of the first measurements of two investigators.

Statistical analysis

Probability values of <0.05 indicated a statistically significant difference. Data are presented as mean ± SD. 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. The Kaplan–Meier method was used to estimate survival as a function of time, and survival difference was analyzed by the log-rank test. Overall survival (OS) was defined as the time between diagnosis and death from any cause. Progression-free survival (PFS) was defined as the time between diagnosis and the first recurrence of the disease (local–regional or distant recurrence). Multivariate analyses were performed using stepwise Cox proportional hazards model to identify independent prognostic factors. Statistical analysis was performed using JMP 8 (SAS, Institute Inc., Cary, NC, USA) for Windows.

Results

Immunohistochemical analysis

Each protein revealed a profile pattern of the unique expression. The immunohistochemical staining of TS, OPRT, DPD, Glut1, HIF-1α, VEGF, CD34, EGFR, p-Akt, p-mTOR and p53 was evaluated for the 160 primary lesions. Figure 1 represents the immunohistochemical staining of TS, OPRT and DPD.

TS, OPRT and DPD were positively expressed in 46% (74/160), 71% (114/160) and 54% (87/160), respectively. A positive rate of TS, OPRT and DPD was recognized in 34% (36/107), 61% (65/107) and 70% (75/107), respectively, of AC and 72% (38/53), 92% (49/53) and 23% (12/53), respectively, of non-AC. The mean scoring of TS, OPRT and DPD was 2.24 ± 0.88, 3.00 ± 1.2 and 2.54 ± 1.05, respectively, in all cases, 1.98 ± 0.88, 2.63 ± 1.15 and 2.86 ± 0.97, respectively, in AC and 2.79 ± 0.61, 3.77 ± 0.94 and 1.88 ± 0.89, respectively, in non-AC. The mean scoring of TS and OPRT expression was significantly higher in non-AC than AC ($P < 0.0001$) but that of DPD expression was significantly lower in non-AC than AC ($P < 0.0001$).

Glut1 was detected in tumor cells and localized predominantly on their plasma membrane. A positive rate of Glut1 expression was recognized in 56%. A positive expression of HIF-1α was predominantly expressed in the cytoplasm with some nuclear staining and was recognized in 76%. The staining pattern of VEGF was uniformly localized in the cytoplasm and/or membrane of neoplastic. The median rate of VEGF positivity was 33% (range, 2–86%), and the value of 33% was chosen as a cutoff point. Positive expression was recognized in 50%. The median number of CD34 was 25 (range, 3–53), and the value of cutoff point was 25. Positive expression of CD34 was seen in 49%. A positive expression of EGFR, p-Akt, and

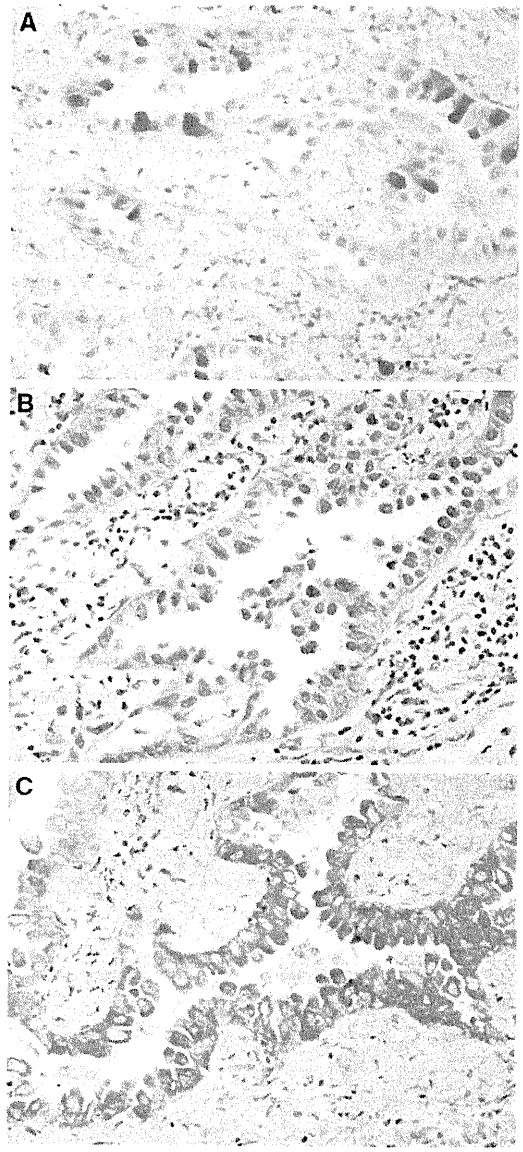

Fig. 1 Immunohistochemical staining of TS, OPRT and DPD in NSCLC. **a** TS is stained in nuclei (score 3). **b** OPRT is stained in mainly cytoplasm (score 4). **c** DPD is stained in nuclei (score 4)

p-mTOR was 56, 48 and 60%, respectively. High expression of p53 was recognized in 46%.

Demographics of patients according to TS, OPRT and DPD

The demographic result of the patients according to these biomarkers is listed in Table 1. In the analysis according to TS expression, histology, lymph node metastases, lymphatic permeation, OPRT, Glut1, HIF-1α, VEGF, CD34, EGFR and p53 yielded a statistically significant difference. In OPRT expression, histology, lymph node metastases, lymphatic permeation, vascular invasion, TS, Glut1, HIF-1α, VEGF, CD34, EGFR, p-mTOR and p53 yielded a statistically significant difference. In DPD expression, stage, histology, Glut1, CD34 and p-Akt yielded a statistically significant difference.

Using Spearman rank correlation, TS expression was significantly correlated with OPRT ($\gamma = 0.5374$, $P < 0.0001$), Glut1 ($\gamma = 0.6260$, $P < 0.0001$), HIF-1α ($\gamma = 0.5366$, $P < 0.0001$), VEGF ($\gamma = 0.6369$, $P < 0.0001$), CD34 ($\gamma = 0.5516$, $P < 0.0001$), EGFR ($\gamma = 0.3854$, $P < 0.0001$) and p-mTOR ($\gamma = 0.2783$, $P = 0.0004$) in all patients. In AC patients, TS expression was significantly correlated with OPRT ($\gamma = 0.5073$, $P < 0.0001$), DPD ($\gamma = 0.2511$, $P = 0.0088$), Glut1 ($\gamma = 0.6577$, $P < 0.0001$), HIF-1α ($\gamma = 0.5320$, $P < 0.0001$), VEGF ($\gamma = 0.6586$, $P < 0.0001$), CD34 ($\gamma = 0.5382$, $P < 0.0001$), EGFR ($\gamma = 0.3211$, $P = 0.0007$), p-Akt ($\gamma = 0.2689$, $P = 0.0049$) and p-mTOR ($\gamma = 0.2307$, $P = 0.0163$). In non-AC patients, however, TS expression was not significantly correlated with all of these biomarkers.

In the analysis according to the tumor differentiation of AC, no statistically significant difference in the mean scoring of TS expression was observed between well differentiated (1.85 ± 0.15) and moderately or poorly differentiated AC (2.06 ± 0.10) ($P = 0.2344$).

Survival analysis according to TS, OPRT and DPD

The 5-year survival rate for OS and PFS was 77.5 and 65.4%, respectively, and the median survival time was not reached. In the analysis of OS and PFS, a statistically significant poorer prognosis was observed in NSCLC ($n = 160$) with the positive expression of TS and DPD. In the analysis according to histology, a statistically significant difference in the OS and PFS was observed in AC patients ($n = 107$) with the positive expression of TS and DPD, but not OPRT. In patients with non-AC ($n = 53$), however, no statistically significant difference was observed according to the expression of TS, OPRT and DPD (Table 2). Figure 2 shows the Kaplan–Meier survival curves in patients with positive and negative for TS expression.

Univariate and multivariate analyses in all patients

Univariate and multivariate analyses of factors which affected the OS and PFS were performed. Univariate analysis demonstrated that a statistically significant difference in the OS was observed in gender, disease stage, TS, DPD, Glut1, HIF-1α, CD34 and EGFR. Multivariate analysis revealed that disease stage was an independent and significant factor to predict a poor prognosis (Table 3). By

Table 1 Different variables according to TS, OPRT and DPD

Variables	TS			OPRT			DPD		
	Positive ($n = 74$)	Negative ($n = 86$)	P value	Positive ($n = 114$)	Negative ($n = 46$)	P value	Positive ($n = 88$)	Negative ($n = 72$)	P value
Age									
≤65/> 65 yrs	35/39	36/50	0.525	48/66	23/23	0.384	38/50	33/39	.0.751
Gender									
Male/female	51/23	46/40	0.052	73/41	24/22	0.211	49/39	48/24	0.194
Stage									
I + II/III	59/15	73/13	0.411	92/22	40/6	0.491	65/23	67/5	**0.002**
Histology									
AC/non-AC	36/38	71/15	**<0.001**	65/49	42/4	**<0.001**	76/12	31/41	**<0.001**
Lymph node metastasis									
Positive/negative	27/47	16/70	**0.012**	39/75	4/42	**<0.001**	26/62	17/55	0.4745
Pleural involvement									
Positive/negative	18/56	22/64	1.000	31/83	9/37	0.420	21/67	19/53	0.718
Vascular invasion									
Positive/negative	33/41	28/58	0.142	54/60	7/39	**<0.001**	34/54	27/45	1.000
Lymphatic permeation									
Positive/negative	37/37	24/62	**0.005**	53/61	8/38	**<0.001**	34/54	27/45	1.000
TS									
Positive/negative	(−)	(−)	(−)	68/46	6/40	**<0.001**	36/52	38/34	0.153
OPRT									
Positive/negative	68/6	46/40	**<0.001**	(−)	(−)	(−)	66/22	48/24	0.293
DPD									
Positive/negative	36/38	52/34	0.153	66/48	22/24	0.293	(−)	(−)	(−)
Glut1									
Positive/negative	62/12	27/59	**<0.001**	79/35	10/36	**<0.001**	38/50	51/21	**<0.001**
HIF-1$^{\alpha}$									
Positive/negative	70/4	52/34	**<0.001**	103/11	19/27	**<0.001**	68/20	54/18	0.852
VEGF									
Positive/negative	56/18	24/62	**<0.001**	75/39	5/41	**<0.001**	40/48	40/32	0.266
CD34									
Positive/negative	56/18	23/63	**<0.001**	70/44	19/27	**0.023**	39/49	50/22	**0.002**
EGFR									
Positive/negative	52/24	37/49	**0.001**	76/38	13/33	**<0.001**	49/39	40/32	1.000
p-Akt									
Positive/negative	36/38	41/45	1.000	58/56	19/27	0.298	57/31	20/52	**<0.001**
p-mTOR									
Positive/negative	48/26	48/36	0.332	79/35	17/29	**<0.001**	53/35	43/29	1.000
P53									
Positive/negative	47/27	26/60	**<0.001**	66/48	7/39	**<0.001**	36/52	37/35	0.205

TS thymidylate synthase, *OPRT* orotate phosphoribosyltransferase, *DPD* dihydropyrimidine dehydrogenase, *AC* adenocarcinoma, *Glut1* glucose transporter 1, *HIF-1α* hypoxia inducible factor-1α, *VEGF* vascular endothelial growth factor, *EGFR* epidermal growth factor receptor, *p-mTOR* phosph-mammalian target of rapamycin

Bold denotes statistically significance value

univariate analysis, a statistically significant difference in the PFS was observed in disease stage, TS, DPD, Glut1, HIF-1α, VEGF, CD34, EGFR and p-Akt. Multivariate analysis demonstrated that disease stage and a positive TS expression were independent prognostic factors to predict a poor outcome (Table 4).

Table 2 Survival analysis according to TS, OPRT and DPD

	TS			OPRT			DPD		
	Positive (%)	Negative (%)	*P* value	Positive (%)	Negative (%)	*P* value	Positive (%)	Negative (%)	*P* value
Total ($n = 160$)									
OS 5-yr rate (%)	67.5	86.1	**0.0002**	71.6	84.7	0.1536	71.9	91.3	**0.0022**
PFS 5-yr rate (%)	48.6	79.1	**<0.0001**	57.9	73.6	0.1409	56.1	86.9	**0.0005**
AC ($n = 107$)									
OS 5-yr rate (%)	61.1	90.1	**<0.0001**	74.6	93.7	0.0572	73.4	90.7	**0.0096**
PFS 5-yr rate (%)	30.5	83.1	**<0.0001**	60.0	78.1	0.2134	51.5	86.1	**0.0008**
Non-AC ($n = 53$)									
OS 5-yr rate (%)	73.6	66.7	0.5561	53.8	77.5	0.1962	70.0	71.7	0.2165
PFS 5-yr rate (%)	65.8	53.3	0.5532	46.2	70.0	0.1371	62.0	62.2	0.2022

TS thymidylate synthase, *OPRT* orotate phosphoribosyltransferase, *DPD* dihydropyrimidine dehydrogenase, *AC* adenocarcinoma, *Non-AC* non-adenocarcinoma, *OS* overall survival, *PFS* progression-free survival, 5-year rate, 5-year survival rate

Bold denotes statistically significance value

Univariate and multivariate analyses according to different variables

In the analysis of patients ($n = 131$) with early stage (stage I or II), the positive expression of TS and DPD was an independent prognostic factor for predicting a poor outcome in the OS and PFS. In patients with stage III ($n = 29$), there was no independent prognostic factors for predicting outcome. Next, we examined the survival analysis according to histological type. In AC patients ($n = 107$), disease staging and TS expression were independent and significant factors to predict a poor prognosis in the OS and PFS. In non-AC patients ($n = 53$), however, TS expression was not significantly associated with poor outcome in the OS and PFS.

Moreover, we compared the different variables between positive and negative TS expression in patients with AC. The positive expression of TS in AC patients was significantly associated with advanced stage, lymph node metastases, vascular invasion, glucose metabolism, hypoxia, angiogenesis, EGFR expression and cell cycle regulator.

Discussion

This is the retrospective study to evaluate the prognostic significance of TS, OPRT and DPD in patients with NSCLC. A multivariate analysis demonstrated the positive TS expression to be independently associated with an increased risk for poor OS and DFS in patients with AC, but not in those with non-AC. TS expression may be a useful marker for predicting postoperative outcome in AC patients. Limited to the analysis of AC patients, TS expression was significantly associated with OPRT, DPD, advanced stage, lymph node metastases, vascular invasion, glucose metabolism (Glut1), hypoxia (HIF-1α), angiogenesis (VEGF and CD34), EGFR/Akt/mTOR pathway and cell cycle regulator (p53). In patients with non-AC (almost SQC), TS expression seemed to be not correlated with outcome and these biomarkers.

High-level TS expression is related to an aggressive tumor phenotype and a poor outcome in a variety of malignant tumors [12, 13]. TS levels are generally lower in adenocarcinoma and in some large cell carcinoma than in squamous cell carcinoma [14]. In lung cancer, TS expression has been described to be higher in neuroendocrine carcinoma of the lung than in squamous cell carcinoma [15]. In our series, TS expression was also significantly higher in non-AC (almost SQC) than in AC. Ceppi et al. [6] had documented higher expression of TS in pulmonary SQC compared with non-squamous histotypes and described that a strong correlation was observed between *TS* mRNA and protein levels. But they had no description about the relationship between TS expression and prognosis in patients with pulmonary SQC. There was a significantly higher proliferative activity in TS-high tumor in NSCLC patients [5]. When stratified according to histology, there was a significant difference in AC, but no difference in SQC. In our study, TS expression in AC patients was closely associated with hypoxia-related protein expression and tumor aggressiveness. Recently, Atkin et al. [8] had described that the direct correlation between TS expression and HIF-1α expression was recognized in primary rectal cancer, and the microenvironmental 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. Thymidine phosphorylase is an enzyme involved in the activation and the metabolism of the

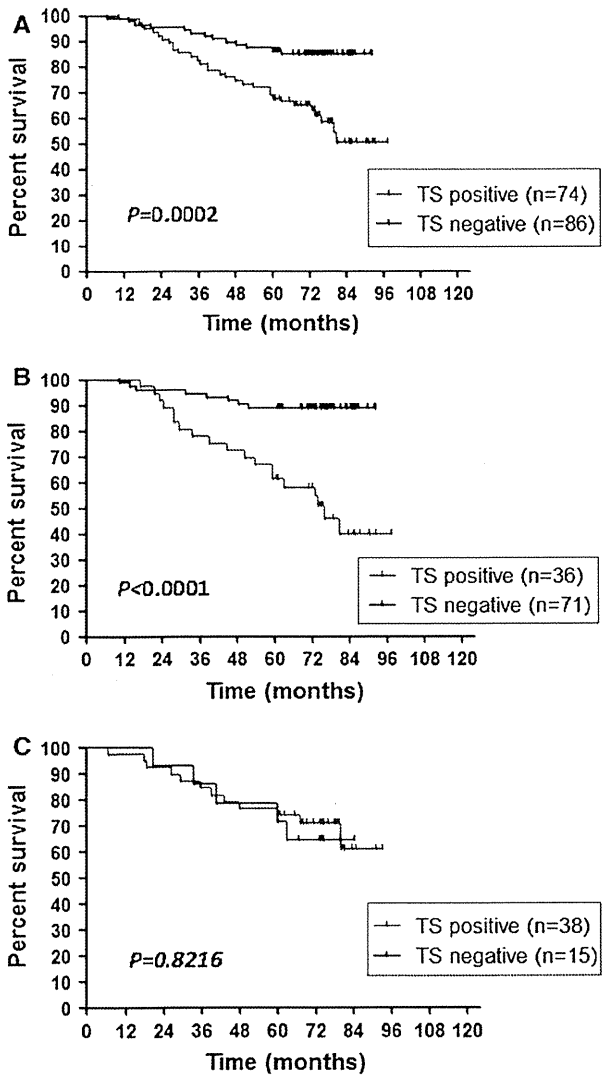

Fig. 2 Overall survival curve according to TS expression in all patients ($n = 160$) (**a**), patients with adenocarcinoma ($n = 107$) (**b**) and patients with squamous cell carcinoma ($n = 53$) (**c**)

fluoropyrimidines, including 5-FU, and previous report documented thymidine phosphorylase induction by hypoxia in a breast carcinoma cell line [16]. These reports including our study suggest that TS expression is related to hypoxia in cancer patients with a histological type of adenocarcinoma. Glut 1 is thought to be a possible intrinsic marker of hypoxia, and the expression of Glut 1 has been found to be regulated by hypoxia in HIF-1α [17, 18]. HIF-1α is considered to support tumor growth by the induction of angiogenesis via the expression of the VEGF and also by high and anaerobic metabolic mechanisms [19]. The overexpression of Glut1 has been shown to be closely related to 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) uptake in human cancer [11]. Previous studies suggest that hypoxic conditions correspond to a higher ^{18}F-FDG uptake [11]. In addition, there are also reports on the relationship between ^{18}F-FDG uptake and the expression of VEGF or MVD [11, 20]. These biomarkers including ^{18}F-FDG uptake have been documented to be closely associated with tumor aggressiveness and outcome in patients with NSCLC. In our study, we found that TS expression was a most powerful prognostic factor among these molecular markers in pulmonary AC patients. In non-AC patients, however, the direct relationship between TS expression and hypoxia was not observed, and TS expression was not related to the outcome after surgery. As TS expression in AC patients was closely correlated with the hypoxic and angiogenic markers (Glut1, HIF-1α and MVD) related to poor outcome, the expression of TS may play an important role for the prognosis and tumor aggressiveness of AC patients, but not non-AC (almost SQC).

It is noteworthy that adjuvant chemotherapy with UFT could improve survival in Japanese patients with completely resected NSCLC [21]. Adjuvant UFT was effective only in patients with early-stage adenocarcinoma, and no significant survival benefit was recognized in patients with SQC. Although these studies did not evaluate the TS expression in tumor specimens, TS inhibitor seems to be effective for the patients with pulmonary adenocarcinoma. Several studies have documented the clinical relevance of low TS expression as a predictor of the response to 5-FU-based chemotherapy and the long survival of the patients with advanced colorectal [22]. The results of these reports suggest that high expression of TS may be resistant to 5-FU-based chemotherapy in human neoplasms. However, it is unclear whether the expression level of TS is closely correlated with the chemoresistance to TS inhibitor regimens. On the other hand, several investigators have documented the relationship between TS expression and 5-FU-based chemotherapy in SQC [22]. In patients with oral SQC, the response to chemotherapy was higher in the group with low TS activity compared with high TS activity [23]. In contrast, high expression of TS in patients with esophageal SQC was associated with a higher response to chemotherapy than low expression of TS [24]. In our series, the patients with SQC had a high TS activity as compared with AC, but the TS expression seemed unlikely to be associated with tumor aggressiveness and metastasis. The prognostic significance of TS expression may be different between NSCLC patients with AC and SQC. Since a high TS expression in NSCLC patients with early-stage AC is associated with poor prognosis, it should be investigated whether the efficacy of UFT as adjuvant therapy is different according to a status of TS expression in patients with early-stage pulmonary AC.

S-1 (Taiho Pharmaceutical Co., Ltd, Tokyo, Japan) is an oral anticancer agent comprised of tegafur (FT), 5-chloro-

Table 3 Univariate and multivariate analyses in the OS

Different variables	Univariate analysis		Multivariate analysis
	5-year survival rate (%)	*P* value	*P value*
Age			
≤65/> 65 years	81.7/74.2	0.1743	
Gender			
Male/female	72.2/85.6	**0.0484**	0.0723
Stage			
I + II/III	84.1/46.4	**<0.0001**	**<0.0001**
Histology			
AC/non-AC	80.3/71.7	0.1494	
TS			
Positive/negative	67.5/86.1	**0.0002**	0.1345
OPRT			
Positive/negative	71.6/84.7	0.1536	
DPD			
Positive/negative	71.9/91.3	**0.0022**	0.1121
Glut1			
Positive/negative	67.4/90.1	**0.0002**	0.1931
HIF-1α			
Positive/negative	72.9/92.1	**0.0105**	0.6478
VEGF			
Positive/negative	73.5/81.3	0.1712	
CD34			
Positive/negative	68.3/86.4	**0.0015**	0.7449
EGFR			
Positive/negative	69.7/87.3	**0.0010**	0.1820
p-Akt			
Positive/negative	74.0/80.7	0.3210	
p-mTOR			
Positive/negative	76.0/79.7	0.6820	
P53			
Positive/negative	73.9/80.5	0.1693	

TS thymidylate synthase, *OPRT* orotate phosphoribosyltransferase, *DPD* dihydropyrimidine dehydrogenase, *AC* adenocarcinoma, *Glut1* glucose transporter 1, *HIF-1α* hypoxia inducible factor-1α, *VEGF* vascular endothelial growth factor, *EGFR* epidermal growth factor receptor, *p-mTOR* phosph-mammalian target of rapamycin

Bold denotes statistically significance value

Table 4 Univariate and multivariate analyses in the PFS

Different variables	Univariate analysis		Multivariate analysis
	5-year survival rate (%)	*P* value	*P value*
Age			
≤ 65/> 65 years	64.8/65.2	0.9477	
Gender			
Male/female	59.8/73.0	0.1488	
Stage			
I + II/III	74.2/21,4	**<0.0001**	**<0.0001**
Histology			
AC/non-AC	65.4/64.2	0.7127	
TS			
Positive/negative	48.6/79.1	**<0.0001**	**0.0409**
OPRT			
Positive/negative	57.9/73.6	0.1409	
DPD			
Positive/negative	56.1/86.9	**0.0005**	0.1655
Glut1			
Positive/negative	51.7/81.7	**<0.0001**	0.1218
HIF-1α			
Positive/negative	57.4/89.5	**0.0002**	0.3529
VEGF			
Positive/negative	56.3/73.8	**0.0253**	0.2632
CD34			
Positive/negative	51.9/77.8	**0.0002**	0.7952
EGFR			
Positive/negative	55.1/77.5	**0.0088**	0.8640
p-Akt			
Positive/negative	57.1/72.3	**0.0341**	0.1022
p-mTOR			
Positive/negative	63.5/67.2	0.4137	
P53			
Positive/negative	57.5/71.3	0.0846	

TS thymidylate synthase, *OPRT* orotate phosphoribosyltransferase, *DPD* dihydropyrimidine dehydrogenase, *AC* adenocarcinoma, *Glut1* glucose transporter 1, *HIF-1α* hypoxia inducible factor-1α, *VEGF* vascular endothelial growth factor, *EGFR* epidermal growth factor receptor, *p-mTOR* phosph-mammalian target of rapamycin

Bold denotes statistically significance value

2,4-dihydroxypyridine (CDHP) and potassium oxonate (Oxo), in a molar ratio of 1:0.4:1 [25]. S-1 is a potent inhibitor of DPD inhibitory fluoropyrimidine (DIF) and is effective against patients with lung, colon and gastric cancers [4, 26]. Recently, S-1 had been documented to be no difference in response and outcome between AC and SQC in advanced NSCLC [27]. However, UFT has been described to be effective in early-stage NSCLC, demonstrating a statistical significant difference between AC and SQC [21]. Our results indicated that TS and DPD expression were a significant prognostic factor in early-stage NSCLC but not in advanced stage NSCLC. The role of TS expression may be different according to not only histological type but also disease stage. Okabe et al. [9] described that inhibition of EGFR induced the down-regulation of TS expression in the experimental study. Our study indicated that TS expression was associated with EGFR/Akt/mTOR pathway in AC patients. The activation of this signaling pathway is related to tumor growth, and therefore, it may play a crucial role on the overexpression of TS in patients with AC. Recently, Fukui et al. [28]

reported the large-scale population study of TS, OPRT and DPD mRNA and protein expression in various cancers from a large number of subjects. High DPD expression was observed in most gastric, lung and pancreatic cancers, and 5-FU-resistant cancers exhibited high expression levels of DPD. Therefore, they described that the tumoral DPD level may be an important factor in predicting the effectiveness of 5-FU-based chemotherapy. The results of our study also demonstrated that a positive expression of DPD was closely related to poor outcome, especially in AC patients. Takeda et al. [29] had documented that a low expression level of TS or of DPD was associated with a better response and longer survival in advanced NSCLC patients treated with chemotherapeutic regimens including S-1, and TS or DPD expression was considered as the 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 five patients other histology. Their preliminary study suggests that TS expression seems to be a predictive marker for S-1 treatment in patients with AC. The expression of TS seems to be closely related to that of DPD in patients with AC. On the other hand, Sun et al. [30] described the clinical significance of TS expression in 193 patients with advanced non-squamous NSCLC treated by pemetrexed-based chemotherapy. Higher response rates for pemetrexed-based chemotherapy were associated with TS negativity (33.7% vs. 14.1, $P = 0.002$), and progression-free survival for pemetrexed-based chemotherapy was significantly longer in groups with TS negativity (4.1 vs. 2.0 months, $P = 0.001$). These reports suggest that low TS expression was associated with better clinical outcome in advanced pulmonary AC patients who were treated with S-1 or pemetrexed-based chemotherapy. Although the majority of our study was not treated by 5-FU or pemetrexed-based chemotherapy, our results indicated that TS protein expression could be a prognostic and predictive marker for predicting outcome after surgical treatment in patients with pulmonary AC. Nowadays, a low TS expression may be a better prognostic marker for predicting a favorable outcome after TS-targeting therapy in patients with advanced pulmonary AC. However, it remains unknown whether TS expression could be a clinical marker for predicting outcome after adjuvant therapy by 5-FU-based chemotherapy such as UFT in completely resected NSCLC patients with early-stage AC. Therefore, further prospective large-scale study is warranted to evaluate the prognostic significance between TS expression and 5-FU-based adjuvant chemotherapy after complete resection.

In conclusion, a positive TS expression was an independent prognostic factor to predict a poor outcome in completely resected NSCLC. When stratified according to histology, TS expression was closely associated with outcome, disease stage, tumor aggressiveness and hypoxia-related protein expression in patients with AC. In patients with early stage, TS and DPD expression seem to be related to a significant factor in predicting prognosis. Although TS expression was significantly higher in non-AC (almost SQC) than AC, the expression of TS in non-AC did not have an important role as the measurement of prognosis or tumor aggressiveness. In future, we will investigate the role of TS expression as a predictive and prognostic marker after 5-FU-based adjuvant chemotherapy in completely resected patients with early-stage pulmonary AC.

Acknowledgments This work was supported in part by Grant 21790793 (K. K) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and National Hospital Organization Policy Based Medical Services. We thank all staffs of pathology department in Shizuoka Cancer Center for their technical assistance of immunohistochemical analysis.

Conflict of interest We, all authors, have no financial or personal relationships with other people or organizations that could inappropriately influence our work.

References

1. Brundage MD, Davies D, Mackillop WJ. Prognostic factors in non-small cell lung cancer: a decade of prognosis. Chest. 2002;122:1037–57.
2. Danenberg PV. Thymidylate synthase: a target enzyme in cancer chemotherapy. Biochim Biophys Acta. 1997;473:73–92.
3. Wada H, Hitomi S, Teramatsu T. Adjuvant chemotherapy after complete resection in non-small cell lung cancer. West Japan Study Group for Lung Cancer Surgery. J Clin Oncol. 1996;14:1048–54.
4. Nakano J, et al. Evaluation of biomarkers associated with 5-FU sensitivity for non-small-cell lung cancer patients postoperatively treated with UFT. Br J Cancer. 2006;95:607–15.
5. Nakagawa T, et al. Expression of thymidylate synthase is correlated with proliferative activity in non-small cell lung cancer (NSCLC). Lung Cancer. 2004;43:145–9.
6. Ceppi P, et al. Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase. Cancer. 2006;107:1589–96.
7. Hashimoto H, et al. Significance of thymidylate synthase gene expression level in patients with adenocarcinoma of the lung. Cancer. 2006;106:1595–601.
8. Atkin GK, et al. The impact of surgically induced ischaemia on protein levels in patients undergoing rectal cancer surgery. Br J Cancer. 2006;95:928–33.
9. Okabe T, et al. Synergistic antitumor effect of S-1 and the epidermal growth factor receptor inhibitor gefitinib in non-small cell lung cancer cell lines: role of gefitinib-induced down-regulation of thymidylate synthase. Mol Cancer Ther. 2008;7:599–606.
10. Mountain CF. Revision in the international system for staging lung cancer. Chest. 1997;11:1710–7.
11. Kaira K, et al. Biologic correlation of 2-[^{18}F]-fluoro-2-deoxy-D-glucose uptake on positron emission tomography in thymic epithelial tumors. J Clin Oncol. 2010;28:3746–53.
12. Shirota Y, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. J Clin Oncol. 2001;19:4298–304.

13. Lenz H, et al. Thymidylate synthase mRNA level in adenocarcinoma of the stomach: a predictor for primary tumor response and overall survival. J Clin Oncol. 1995;14:176–82.
14. Ceppi P, et al. Thymidylate synthase expression in gastroenteropancreatic and pulmonary neuroendocrine tumors. Clin Cancer Res. 2008;14:1059–64.
15. Ibe T, et al. High-grade neuroendocrine carcinoma of the lung shows increased thymidylate synthase expression compared to other histotypes. J Surg Oncol. 2010;102:11–7.
16. Griffiths L, et al. The influence of oxygen tension and pH on the expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human breast tumor cells grown in vitro and in vivo. Cancer Res. 1997;57:570–2.
17. Vleugel MM, et al. Differential prognostic impact of hypoxia induced and diffuse HIF-1 alpha expression in invasive breast cancer. J Clin Pathol. 2005;58:172–7.
18. Elson DA, et al. Coordinate up-regulation of hypoxia inducible factor (HIF)-1a and HIF-1 target genes during multi-stage epidermal carcinogenesis and wound healing 1. Cancer Res. 2000;60:6189–95.
19. Ryan HE, et al. Hypoxia-inducible factor-1α is a positive factor in solid tumor growth. Cancer Res. 2000;60:4010–5.
20. Kaira K, et al. Correlation of angiogenesis with ^{18}F-FMT and ^{18}F-FDG uptake in non-small cell lung cancer. Cancer Sci. 2009;100:753–8.
21. Hamada C, et al. Meta-analysis of postoperative adjuvant chemotherapy with tegafur-uracil in non-small-cell lung cancer. J Clin Oncol. 2005;23:4999–5007.
22. Ishihama H, et al. Comparison of 5-fluorouracil-related gene expression levels between adenocarcinomas and squamous cell carcinomas of the lung. Jpn J Clin Oncol. 2009;39:33–6.
23. Harada K, Kawashima Y, Yoshida H, Sato M. Thymidylate synthase expression in oral squamous cell carcinoma predicts response to S-1. Oncol Rep. 2006;15:1417–23.
24. Lee S, et al. Thymidylate synthase, thymidine phosphorylase, and excision repair-complementation group 1 expression as predictive markers of capecitabine plus cisplatin chemotherapy as first-line treatment for patients with advanced oesophageal squamous cell carcinoma. Br J Cancer. 2010;7:845–51.
25. Shirasaka T, et al. Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs. 1996;7:548–57.
26. Kawahara M, et al. Phase II study of S-1, a novel oral fluoropyrimidine, in advanced non-small-cell lung cancer. Br J Cancer. 2001;85:939–43.
27. Yamamoto N, et al. Pooled analysis of S-1 trials in non-small cell lung cancer according to histological type. Anticancer Res. 2010;30:2985–90.
28. Fukui Y, Oka T, Nagayama S, Danenberg PV, Danenberg KD, Fukushima M. Thymidylate synthase, dihydropyrimidine dehydrogenase, orotate phosphoribosyltransferase mRNA and protein expression levels in solid tumors in large scale population analysis. Int J Mol Med. 2008;22:709–16.
29. Takeda M, Okamoto I, Hirabayashi N, Kitano M, Nakagawa K. Thymidylate synthase and dihydropyrimidine dehydrogenase expression levels are associated with response to S-1 plus carboplatin in advanced non-small cell lung cancer. Lung Cancer. 2011;73:103–9.
30. Sun JM, Han J, Ahn JS, Park K, Ahn MJ. Significance of thymidylate synthase and thyroid transcription factor 1 expression in patients with nonsquamous non-small cell lung cancer treated with pemetrexed-based chemotherapy. J Thorac Oncol. 2011;6:1392–9.

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EURR-5699; No. of Pages 7
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European Journal of Radiology xxx (2011) xxx–xxx

Contents lists available at SciVerse ScienceDirect

European Journal of Radiology

journal homepage: www.elsevier.com/locate/ejrad

^{18}F-FDG uptake on PET in primary mediastinal non-thymic neoplasm: A clinicopathological study

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ARTICLE INFO

Article history:
Received 19 July 2011
Received in revised form
19 September 2011
Accepted 20 September 2011

Keywords:
^{18}F-FDG PET
Mediastinal neoplasm
Biological correlation
Glut1
HIF-1α
Glucose metabolism

ABSTRACT

Background: The usefulness of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) has been investigated in thymic epithelial tumors. However, little is known about PET imaging of ^{18}F-FDG in primary non-thymic mediastinal neoplasms. The aim of this study is to explore the clinicopathological significance of ^{18}F-FDG PET in primary mediastinal (non-thymic) neoplasms.
Methods: Twenty-one patients with mediastinal neoplasms who underwent ^{18}F-FDG PET before treatment were included in this study. Tumor sections were stained by immunohistochemistry for glucose transporter 1 (Glut1); glucose transporter 3 (Glut3); hypoxia-inducible factor-1 alpha (HIF-1α); hexokinase I; vascular endothelial growth factor (VEGF); microvessels (CD34); epidermal growth factor receptor (EGFR); Akt/mTOR signaling pathway (p-Akt and p-mTOR); cell cycle control (p53).
Results: Seventeen of 21 patients were imaged on PET system using ^{18}F-FDG, but 4 patients with a histology of cyst showed nothing abnormal in PET scans. The histology of the resected tumors was as follows: 6 schwannoma, 3 teratoma, 4 cyst, 3 sarcoma, 1 undifferentiated carcinoma, 1 seminoma, 1 mediastinal goiter, 1 ganglioneuroma, and 1 Hodgkin lymphoma. ^{18}F-FDG uptake was significantly correlated with Glut1, HIF-1α, EGFR, p-Akt and p-S6K. These biomarkers were highly expressed in schwannoma, teratoma and high grade malignancies, whereas all patients with cyst and ganglioneuroma had no positive expression of these biomarkers. High uptake of ^{18}F-FDG was significant associated with Glut1, VEGF, EGFR, p-Akt, p-S6K and tumor maximal size.
Conclusion: The amount of ^{18}F-FDG uptake in primary mediastinal non-thymic neoplasms is determined by the presence of glucose metabolism (Glut1), hypoxia (HIF-1α) and upstream components of HIF-1α (EGFR, p-Akt and p-S6K).

1. Introduction

Primary mediastinal tumors are rare diseases, representing approximately 3% of the tumors within the thorax [1,2]. They can originate from any organ or tissue but most commonly arise from thymic, neurogenic, lymphatic, germinal or mesenchymal tissues. Primary tumors of the mediastinum are a heterogenous group of tumors, ranging from relatively benign tumors to aggressive malignancies.

Recently, the usefulness of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) for the diagnosis of cancer has been investigated in many studies [3–5]. Determination of malignant lesions with ^{18}F-FDG PET is based on the glucose metabolism. The overexpression of glucose transporter 1 (Glut1) has been shown to be closely related to ^{18}F-FDG uptake in human cancer [3–5]. Glucose phosphorylation enzyme (Hexokinase) is also known to play an important subtype for glucose metabolism in cancer cells [4]. Glucose-6-phosphatase is decreasing by the increased concentrations of hexokinase, and the acceleration of glucose phosphorylation results in increased glucose consumption. Glut 1 is thought to be a possible intrinsic marker of hypoxia, and the expression of Glut 1 has been found to be regulated by hypoxia in a

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doi:10.1016/j.ejrad.2011.09.017

Please cite this article in press as: Kaira K, et al. ^{18}F-FDG uptake on PET in primary mediastinal non-thymic neoplasm: A clinicopathological study. Eur J Radiol (2011), doi:10.1016/j.ejrad.2011.09.017

hypoxia-inducible factor (HIF)-1-dependent way [3,6,7]. Previous studies suggest that hypoxic conditions correspond to a higher ^{18}F-FDG uptake [3,6,7]. In addition, several researchers described the relationship between ^{18}F-FDG uptake and the expression of vascular endothelial growth factor (VEGF) or micro-vessel density (MVD) [8,9]. HIF-1α is considered to support tumor growth by the induction of angiogenesis via the expression of the VEGF and also by high and anaerobic metabolic mechanisms [10]. Recent preliminary report demonstrated that ^{18}F-FDG PET could be a valuable tool for assessing the effects of the mammalian target of rapamycin (mTOR) inhibition in lung cancer patients [11]. mTOR is a downstream component of the PI3K/AKT pathway involved in the regulation of cell proliferation, angiogenesis, and metabolism. However, there is no report about the relationship between ^{18}F-FDG uptake within tumor cells and PI3K/AKT/mTOR signaling pathway in human neoplasms. As many factors can influence the extent of ^{18}F-FDG uptake, the underlying mechanisms for ^{18}F-FDG accumulation are still a matter of debate in various human neoplasms.

Recently, we reported the biological correlation of ^{18}F-FDG uptake on PET in thymic epithelial tumors as primary mediastinal tumors [3]. Our report demonstrated that the amount of ^{18}F-FDG uptake in thymic epithelial tumors is determined by the presence of glucose metabolism (Glut1), hypoxia (HIF-1α), angiogenesis (VEGF and MVD) and cell cycle regulator (p53). However, there is still no data about the possible mechanisms for ^{18}F-FDG uptake in patients with primary mediastinal tumors excluding thymic epithelial tumors. Therefore, we conducted ^{18}F-FDG PET studies and immunohistochemical analyses in patients with primary mediastinal non-thymic tumors.

2. Materials and methods

2.1. Patients

Between November 2002 and August 2008, we analyzed 78 consecutive patients with primary mediastinal tumors who underwent ^{18}F-FDG PET and received a surgical resection at Shizuoka Cancer Center. The patients with 54 thymic epithelial tumors and 3 mediastinal metastases from extrathoracic malignancies were excluded from this study. Therefore, a total of 21 patients with mediastinal neoplasms were included in this study.

None of the patients had insulin-dependent diabetes, and the serum glucose levels in all patients just before ^{18}F-FDG PET infection were less than 120 mg/dL. The study protocol was approved by the institutional review board.

2.2. ^{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. 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. The region of interest (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. Finally, the T/M ratio, which is the ratio of the peak SUV of the tumor to the mean SUV of the mediastinum, was determined for each patient.

2.3. Immunohistochemical staining

Immunohistochemical staining was performed according to the procedure described in the previous reports [3,12]. The following antibodies were used: a rabbit polyclonal antibody against GLUT1 (AB15309, Abcam, Tokyo, Japan, 1:200 dilution); a rabbit polyclonal antibody against GLUT3 (AB15311, Abcam, Tokyo, Japan, 1:100 dilution); a rabbit monoclonal antibody against hexokinase I (AB55144, Abcam, Tokyo, Japan, 1:200 dilution); a mouse monoclonal antibody against HIF-1α (NB100-123, Novus Biologicals, Inc., Littleton, 1:50 dilution); a murine monoclonal antibody against MIB-1 (Dako, Denmark, 1:40 dilution); a monoclonal antibody against VEGF (Immuno-Biological Laboratories Co.,Ltd., Japan, 1:300 dilution); a mouse monoclonal antibody against CD34 (Nichirei, Tokyo, Japan, 1:800 dilution); a mouse monoclonal antibody against p53 (D07; DAKO, 1:50 dilution); a mouse monoclonal antibody against EGFR (Novovastra laboratories Ltd., Newcastle, UK, 1:100 dilution); a rabbit polyclonal antibody against phosph-Akt (Abcam, Tokyo, Japan, 1:200 dilution); a rabbit monoclonal antibody against phosph-mTOR (Cell signaling, 80 dilution); a rabbit monoclonal antibody against phosph-S6K (Cell signaling, 100 dilution).

The expression of Glut1, Glut3 and EGFR was considered positive if distinct membrane staining was present. Five fields (400×) were analyzed to determine the frequency of the HIF-1α stained nuclei and hexokinase I stained cytoplasm. For Glut1, Glut3, EGFR, HIF-1α and hexokinase I, a semi-quantitative scoring method was used: 1 = <10%, 2 = 10–25%, 3 = 25–50%, 4 = 51–75% and 5 = >75% of cells positive. 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 1000 neoplastic cells. The number of CD34-positive vessels was counted in four selected hot spots in a 400× field (0.26 mm^2 field area). Microvessel density (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 previous report [12], p53 expression in more than 10% of tumor cells was defined as high expression.

p-AKT, p-mTOR and p-S6K were considered positive if membranous and/or cytoplasmic staining was present. For p-AKT, p-mTOR and p-S6K, a semi-quantitative scoring method was used: 1 = <10%, 2 = 10–25%, 3 = 25–50%, 4 = 51–75% and 5 = >75% of cells positive. The tumors in which stained tumor cells made up more than 25% of the tumor were graded as positive.

Sections were assessed using a light microscopic (E330-ADU1.2X, OLYMPUS, Japan) in a blinded fashion by at least two of the authors.

2.4. 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.

3. Results

3.1. Patient characteristics

Patient's characteristics are listed in Table 1. The median age of the patients was 59 years (range, 12–82 years). Eleven patients

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Table 1
Patient's characteristics.

No.	Age/sex	PS	Smoking history	Tumor size (mm)	T/M ratio	Histology
1	59F	0	Yes	27	1.87	Schwannoma
2	82M	0	Yes	63	(–)	Bronchogenic cyst
3	79M	1	Yes	139	(–)	Pericardial cyst
4	76M	0	Yes	30	(–)	Bronchogenic cyst
5	56M	0	Yes	43	4.25	Synovial sarcoma
6	17M	2	No	140	1.58	Mature teratoma
7	56F	0	No	21	(–)	Bronchogenic cyst
8	67M	0	Yes	70	9.28	Undifferentiated carcinoma
9	25M	0	Yes	12	2.52	Seminoma
10	80F	0	No	37	2.12	Mediastinal goiter
11	45F	0	No	28	3.38	Schwannoma
12	59M	0	Yes	42	1.64	Teratoma
13	72M	0	Yes	26	1.45	Schwannoma
14	45F	0	No	160	3.31	Liposarcoma
15	62F	0	No	20	1.74	Schwannoma
16	60F	0	No	46	3.12	Mature teratoma
17	12M	0	N0	105	1.05	Ganglioneuroma
18	33F	0	N0	54	1.75	Schwannoma
19	45M	0	Yes	17	1.59	Schwannoma
20	67F	0	No	65	2.58	Sarcoma
21	71F	1	Yes	45	6.48	Hodgkin lymphoma

Abbreviations: M, male; F, female; T/M ratio, the ratio of the peak SUV of the tumor to the mean SUV of the mediastinum; Tumor size, maximal tumor size.

were men and 10 were women. Performance status (PS) and smoking history were as follows: 18 patients with PS 0; 2 patients with PS 1; 1 patients with PS 2; 11 patients with smoker; 10 patients with never smoker. The maximal tumor size of resected pulmonary nodules ranged from 12 to 140 mm (median, 43 mm). The histology of the resected tumors was as follows: 6 schwannoma, 3 teratoma, 4 cyst, 3 sarcoma, 1 undifferentiated carcinoma, 1 seminoma, 1 mediastinal goiter, 1 ganglioneuroma, and 1 Hodgkin lymphoma.

3.2. ^{18}F-FDG PET findings

Of 21 patients, 17 patients were imaged on PET system using ^{18}F-FDG (Table 1). All of 4 patients with cyst showed nothing abnormal in PET scans. The mean value (mean and standard deviation) of T/M ratio was 3.06 ± 0.52 (range, 1.1–9.3). The maximal size of the primary tumor ($n = 17$) was not significantly correlated with ^{18}F-FDG uptake ($p = 0.278$).

The median value of T/M ratio was 2.51, therefore, a median value of 2.5 was used as the cutoff T/M ratio in the following analyses, and the T/M ratio in more than 2.5 was defined as high uptake.

3.3. Immunohistochemical analysis

Glut1, Glut3, Hexokinase I, HIF-1α, VEGF, CD34, EGFR, p-Akt, p-mTOR, p-S6K and p53 immunohistochemical staining were evaluated for the surgically resected 21mediastinal tumors (Figs. 1 and 2). Glut1 and Glut3 were detected in tumor cells and localized predominantly on their plasma membrane. A positive rate of Glut1 and Glut3 expression was recognized in 38.1% and 23.1%, respectively. A positive expression of HIF-1α was predominantly expressed in the cytoplasm with some nuclear staining, and was recognized in 47.6%. A positive expression of hexokinase I was expressed in the cytoplasm and/or membrane of neoplastic, and was recognized in 42.9%. The staining pattern of VEGF was uniformly localized in the cytoplasm and/or membrane of neoplastic. The median rate of VEGF positivity was 10% (range, 1–40%), and the value of 10% was chosen as a cutoff point. High expression was recognized in 38.1%. The median numbers of CD34 was 14 (1–45), and the value of 14 was chosen as a cutoff point. High expression of CD34 was seen in 33.3%. The positive rate of p-Akt, mTOR and p-S6K was recognized in 33.3%, 14.3% and 52.3%, respectively. The incidence of p53 positivity was 14.3%.

Next, we examined the expression of these immunohistochemical markers according to the histology of mediastinal tumors (Table 2). The positive expression of these biomarkers was observed in schwannoma, teratoma and other thoracic malignancies. But, all patients with cyst and ganglioneuroma had no positive expression of these biomarkers.

3.4. Relationship between ^{18}F-FDG uptake and different variables

The results of the statistical correlation between T/M ratio and different variables are listed in Table 3. The resected specimens of 17 patients with visible imaging on PET were correlated with the degree of ^{18}F-FDG uptake. Using Spearman rank correlation, the T/M ratio in mediastinal tumors was significantly correlated with the expression of Glut1, HIF-1α, EGFR, p-Akt and p-S6K (Fig. 3).

3.5. Comparison of ^{18}F-FDG uptake and different variables

The results of the statistical comparison between ^{18}F-FDG uptake and different variables are listed in Table 4. We analyzed 21 mediastinal neoplasms including 4 patients without visible imaging on PET. Glut1, VEGF, EGFR, p-Akt, p-S6K and tumor size yield a statistically significant positive correlation.

4. Discussion

This is the retrospective study to evaluate the biologic correlation of ^{18}F-FDG uptake and the expression of biomarkers such as Glut1, Gllut3, hexokinase I, HIF-1α, VEGF, CD34, EGFR, p-Akt, p-mTOR, p-S6K and p53 in primary mediastinal non-thymic neoplasms. Glucose transporter (Glut1), hypoxia (HIF-1α), EGFR and p-Akt were closely associated with ^{18}F-FDG uptake. These biomarkers were observed in schwannoma, teratoma and other thoracic malignancies. The significant factors of a high ^{18}F-FDG uptake were Glut1, VEGF, EGFR, p-Akt, p-S6K and tumor maximal size.

Of the 254 primary mediastinal masses reported, thymic neoplasms have been described to be most common [13]. We found that ^{18}F-FDG uptake in thymic neoplasms was closely associated with the expression of Glut1, HIF-1α, VEGF, CD34 and p53 [13]. However, there is still no data about the relationship between ^{18}F-FDG uptake and biomarkers in non-thymic mediastinal neoplasms. Since these diseases are a rare and hetergenous group, it may be

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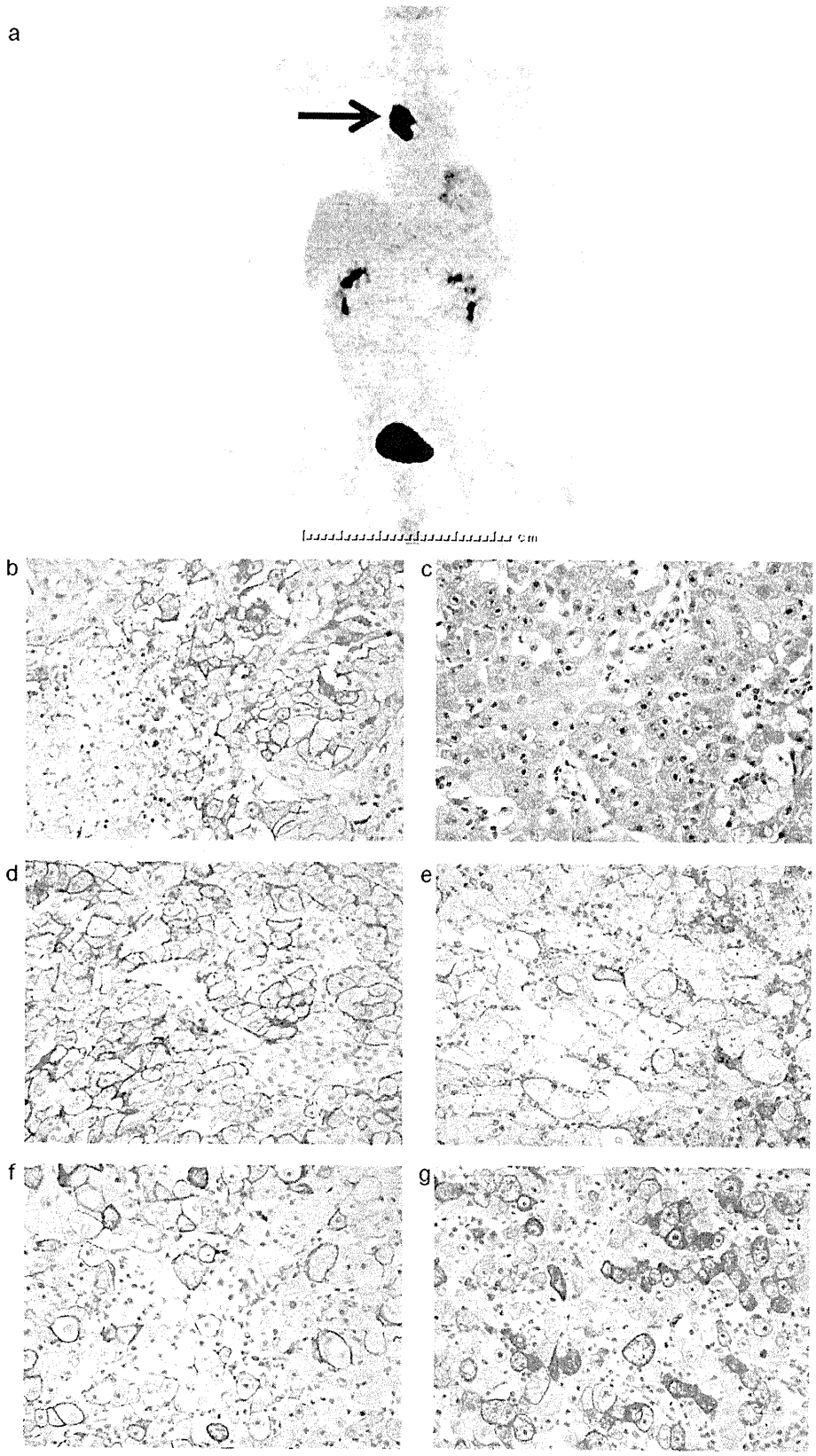

Fig. 1. PET imaging and immunohistochemical staining in a 62-year-old man with undifferentiated carcinoma in the mediastinum. PET showed increased ^{18}F-FDG accumulation in the mediastinal lesion (black arrow) (A). Immunohistochemical analysis showed positive staining of Glut1 (B), HIF-1α (C), EGFR (D), p-Akt (E), p-S6K (F) and p-mTOR (G).

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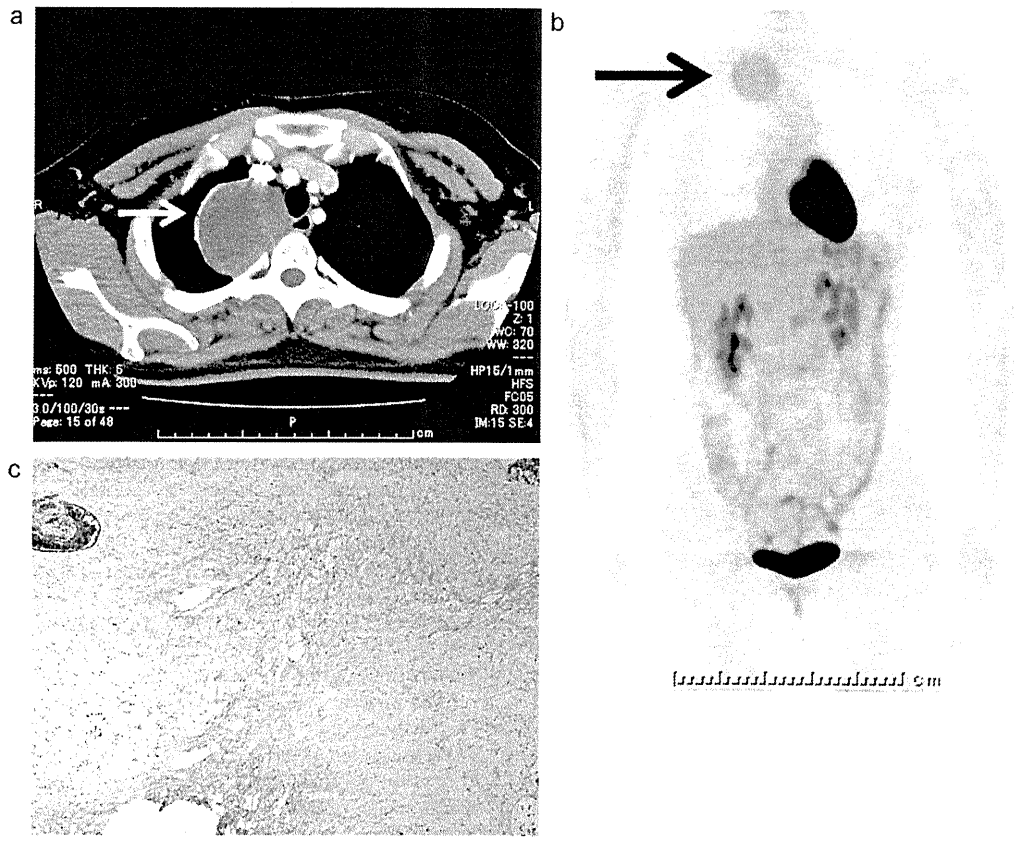

Fig. 2. PET imaging and immunohistochemical staining in a 59-year-old woman with schwannoma in the upper mediastinum. Chest computed tomography showed a mass in the upper mediastinum (A) and PET showed weak uptake of ^{18}F-FDG in the corresponding lesion (black arrow) (B). Immunohistochemical analysis of the resected mass showed positive staining of Glut1 (C).

difficult to investigate the immunohistochemical study of ^{18}F-FDG uptake on PET. Our results suggest that glucose metabolism and hypoxia play an important role on the uptake of ^{18}F-FDG in primary mediastinal non-thymic neoplasms, which is associated with the upstream component of Glut1/HIF-1α pathway such as Akt or EGFR. Since there is only our study assessing the expression of glucose metabolism and hypoxia including signal pathway in primary mediastinal neoplasms, further investigation is warranted.

Recently, ^{18}F-FDG PET analysis of schwannoma has been reported, and ^{18}F-FDG uptake showed a positive correlation with the tumor size and angiogenesis [14]. All of 26 patients with schwannoma showed negative Glut1, 4 patients (15%) positive Glut3, 15 patients (58%) positive Hexokinase, 9 patients (35%) positive VEGF and 9 patients (35%) positive MVD. However, there was no significant correlation between ^{18}F-FDG uptake and the expression of Glut1, Glut3 and Hexokinase. In our study, Glut1 expression was recognized in 3 (50%) of 6 patients with schwannoma, although our study includes a small sample size of schwannoma. Previous report also described that all tumors of schwannoma showed a positive study in ^{18}F-FDG PET, which was corresponding to our results. Therefore, the expression of Glut1, hexokinase, HIF-1α, and VEGF is considered to be necessary for ^{18}F-FDG accumulation within schwannoma cells. The discrepancy between our study and previous report [14] may be due to the differentiation of protocols and antibodies in order for immunohistochemistry.

In patients with bone and soft tissue sarcomas, Glut1 expression has been described to be closely associated with ^{18}F-FDG uptake [15]. Sarcoma patients have an enhanced glucose metabolism which is correlated with tumor grade. Moreover, the significant correlation between Glut1 expression and ^{18}F-FDG uptake has been also reported to be observed in patients with lymphoma [16]. But, several researchers described that intensity of lymphoma on ^{18}F-FDG PET is not significantly associated with the expression of hexokinase and Glut3 [16,17]. However, little is known about the relationship between ^{18}F-FDG uptake and these biomarkers in the metastatic sites secondary to sarcoma or lymphoma. In the present study, Glut1 and hexokinase were highly expressed in the histology of Hodgkin lymphoma and sarcoma. However, these diseases in our study are small sample size, and we could not investigate the correlation between ^{18}F-FDG uptake and Glut1 expression.

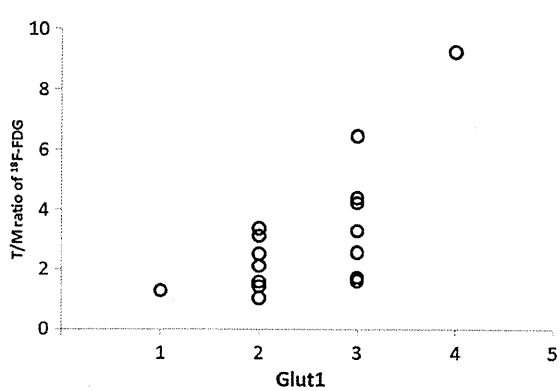

Fig. 3. Significant correlation was found between the T/M ratio of ^{18}F-FDG and scoring of Glut1 expression (Spearman's rank correlation coefficient $\gamma = 0.5965$, $p = 0.012$).

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Table 2
Positive rate of immunohistochemical markers according to histology of primary mediastinal tumors.

Histology (n = 21)	Glut1	Glut3	Hexo I	HIF-1α	VEGF	CD34	EGFR	p-Akt	p-mTOR	p-S6K	p53
Schwannoma (n = 6)	50% (3/6)	33% (2/6)	83% (5/6)	100% (6/6)	50% (3/6)	17% (1/6)	17% (1/6)	17% (1/6)	0% (0/6)	50% (3/6)	33% (2/6)
Teratoma (n = 3)	67% (2/3)	0% (0/3)	33% (1/3)	0% (0/3)	0% (0/3)	67% (2/3)	67% (2/3)	33% (1/3)	33% (1/3)	33% (1/3)	0% (0/3)
Cyst (n = 4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/4)
Sarcoma (n = 3)	33% (1/3)	50% (2/4)	67% (2/3)	33% (1/3)	67% (2/3)	100% (3/3)	67% (2/3)	67% (2/3)	0% (0/3)	100% (3/3)	0% (0/3)
Undifferential carcinoma (n = 1)	100% (1/1)	0% (0/1)	100% (1/1)	100% (1/1)	100% (1/1)	0% (0/1)	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Seminoma (n = 1)	0% (0/1)	0% (0/1)	100% (1/1)	100% (1/1)	100% (1/1)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/1)	100% (1/1)	0% (0/1)
Mediastinal goiter (n = 1)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/1)
Ganglioneuroma (n = 1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)
Hodgkin lymphoma (n = 1)	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)	0% (0/1)
Total positive rate (%)	38%	23%	43%	48%	38%	33%	29%	33%	14%	52%	14%

Abbreviations: Glut1, glucose transporter 1; Glut3, glucose transporter 3; Hexo I, hexokinase I; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor; mTOR, mammalian target of rapamycin.

Table 3
Relationship between ^{18}F-FDG uptake and biomarkers.

Biomarkers	Spearman γ	95% confidence interval	p-Value
Glut1	0.5965	0.1471–0.8471	0.0115
Glut3	0.0362	−0.4647 to 0.5195	0.8903
Hexokinase I	0.4047	−0.1097 to 0.7481	0.1071
HIF-1α	0.5400	0.0646–0.8156	0.0253
VEGF	0.3559	−0.1657 to 0.7219	0.1609
CD34	0.3408	−0.1824 to 0.7136	0.1808
EFGR	0.5973	0.1484–0.8421	0.0013
p-Akt	0.6170	0.1788–0.8510	0.0083
p-mTOR	0.2728	−0.2539 to 0.6747	0.2895
p-S6K	0.5580	0.0902–0.8241	0.0199

Abbreviations: Glut1, glucose transporter 1; Glut3, glucose transporter 3; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor; mTOR, mammalian target of rapamycin.

A previous *in vivo* study demonstrated that changes in ^{18}F-FDG uptake during mTOR inhibitor correlated with p-Akt activation and Glut1 expression [18]. This report suggests that ^{18}F-FDG PET correlates with Akt pathway activity in neoplasm. EGFR is an upstream component of the PI3K/AKT pathway, and our data suggests that not only p-Akt but also EGFR activity is closely associated with the mechanism of ^{18}F-FDG uptake within tumor cells. As this association may be different according to the histological type of primary mediastinal tumors, further study is warranted.

The present study has several limitations. Firstly, our population was a small sample size, including a heterogeneous group of tumors. Non-thymic mediastinal neoplasms were rare tumors, thus, the present study warrants a larger multicenter study. Another limitation is that our study includes various histological types, therefore, the biological correlation of ^{18}F-FDG uptake in one histological type seems to be unclear. Moreover, it is unclear whether ^{18}F-FDG uptake is associated with outcome in primary mediastinal neoplasms. In thymic epithelial tumors, we reported that a high uptake of ^{18}F-FDG is significantly related to poor outcome [13].

In conclusion, glucose metabolism (Glut1), hypoxia (HIF-1α), EGFR and p-Akt play an important role on ^{18}F-FDG uptake in primary mediastinal non-thymic neoplasms. These biomarkers were highly expressed in schwannoma, teratoma and high grade malignancies, whereas all patients with cyst and ganglioneuroma had no positive expression of these biomarkers. Our results suggest that ^{18}F-FDG uptake was useful for predicting the grade of malignancy.

Table 4
Relationship between T/M ratio of ^{18}F-FDG uptake and different variables.

Different variables	T/M ratio of ^{18}F-FDG uptake		
	High (n = 9)	Low (n = 12)	p-Value
Age (≤65/>65 years)	6/3	10/2	0.6108
Gender (male/female)	3/6	5/7	1.0000
Smoking history (yes/no)	4/5	4/8	0.6731
Maximal size of tumor (≤43/>43 mm)	6/3	2/10	0.0318
Glut 1 (positive/negative)	6/3	2/10	0.0318
Glut 3 (positive/negative)	3/6	2/10	0.6018
Hexokinase I (positive/negative)	6/3	3/9	0.0872
HIF-1α (positive/negative)	6/3	4/8	0.1984
VEGF (positive/negative)	6/3	2/10	0.0318
CD34 (positive/negative)	5/4	2/10	0.1588
EGFR (positive/negative)	6/3	0/12	0.0015
p-Akt (positive/negative)	6/3	1/11	0.0158
p-mTOR (positive/negative)	3/6	0/12	0.0632
p-S6K (positive/negative)	8/1	3/9	0.0075
p53 (positive/negative)	2/7	1/11	0.5534

Abbreviations: Glut1, glucose transporter 1; Glut3, glucose transporter 3; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor; mTOR, mammalian target of rapamycin.

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But, a large scale study is necessary for the confirmation of our results.

Conflict of interest statement

We, all authors, have no financial or personal relationships with other people or organizations that could inappropriately influence our work.

Acknowledgements

This work was supported in part by Grant 21790793 (K.K.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and National Hospital Organization Policy Based Medical Services.

We thank all staffs of pathology department in Shizuoka Cancer Center for their technical assistance of immunohistochemical analysis.

References

[1] Rubush JL, Gardner IR, Boyd WC, Ehrenhaft JL. Mediastinal tumors: review of 186 cases. J Thorac Cardiovasc Surg 1973;65:216–22.
[2] Wongsangiem M, Tangthangtham A. Primary tumors of the mediastinum: 190 cases analysis (1975–1995). J Med Assoc Thai 1996;79:689–97.
[3] Kaira K, Endo M, Abe M, et al. Biologic correlation of 2-[^{18}F]-fluoro-2-deoxy-D-glucose uptake on positron emission tomography in thymic epithelial tumors. J Clin Oncol 2010;28:3746–53.
[4] Mameda M, Higashi T, Kitaichi M, et al. [^{18}F] FDG uptake and PCNA, Glut-1, and hexokinase-II expressions in cancers and inflammatory lesions of the lung. Neoplasia 2005;7:369–79.
[5] Higashi K, Ueda Y, Sakurai A, et al. Correlation of Glut-1 glucose transporter expression with [^{18}F] FDG uptake in non-small cell lung cancer. Eur J Nucl Med 2000;27:1778–85.
[6] Elson DA, Ryan HE, Snow JW, Johnson R, Arbeit JM. Coordinate up-regulation of hypoxia inducible factor (HIF)-1a and HIF-1 target genes during multi-stage epidermal carcinogenesis and wound healing 1. Cancer Res 2000;60:6189–95.
[7] van Baardwijk A, Dooms C, van Suylen RJ, et al. The maximum uptake of ^{18}F-deoxyglucose on positron emission tomography scan correlates with survival, hypoxia inducible factor-1α and GLUT-1 in non-small cell lung cancer. Eur J Cancer 2007;43:1392–8.
[8] Guo J, Higashi K, Ueda Y, et al. Microvessel density: correlation with ^{18}F-FDG uptake and prognosis impact in lung adenocarcinomas. J Nucl Med 2006;47:419–25.
[9] Kaira K, Oriuchi N, Shimizu K, et al. Correlation of angiogenesis with ^{18}F-FMT and ^{18}F-FDG uptake in non-small cell lung cancer. Cancer Sci 2009;100:753–8.
[10] Ryan HE, Polni M, McNulty W, et al. Hypoxia-inducible factor-1α is a positive factor in solid tumor growth. Cancer Res 2000;60:4010–5.
[11] Nogová L, Boellaard R, Kobe C, et al. Downregulation of ^{18}FDG uptake in PET as an early pharmacodynamic effect in treatment of non-small cell lung cancer with the mTOR inhibitor everlimus. J Nucl Med 2009;50:1815–9.
[12] Riedl CC, Akhurst T, Larson S, et al. ^{18}F-FDG PET scanning correlates with tissue markers of poor prognosis and predicts mortality for patients after liver resection for colorectal metastases. J Nucl Med 2007;48:771–5.
[13] Azarow KS, Pearl RH, Zurcher R, Edwards FH, Cohen AJ. Primary mediastinal masses: a comparison of adult and pediatric populations. J Thorac Cardiovasc Surg 1993;106:67–72.
[14] Hamada K, Tomita Y, Qiu Y, et al. ^{18}F-FDG PET analysis of schwannoma: increase of SUVmax in the delayed scan is correlated with elevated VEGF/VPF expression in the tumors. Skeletal Radiol 2009;38:261–6.
[15] Tateishi U, Yamaguchi U, Seki K, Terauchi T, Arai Y, Hasegawa T. Glut1 expression and enhanced glucose metabolism are associated with tumour grade in bone and soft tisuue sarcomas: a prospective evaluation by [^{18}F] fluorodexyglucose positron emission tomography. Eur J Nucl Med Mol Imaging 2006;33:683–91.
[16] Khandani AH, Dunphy CH, Meteesatien P, Dufault DL, Ivanovic M, Shea TC. Glut1 and Glut3 expression in lymphoma and their association with tumor intensity on ^{18}F-fluorodexyglucose positron emission tomography. Nucl Med Commun 2009;30:594–601.
[17] Shim HK, Lee WW, Park SY, Kim H, Kim SE. Relationship between FDG uptake and expressions of glucose transporter type 1, type 3, and hexokinase-II and Reed–Sternberg cells of Hodgkin lymphoma. Oncol Res 2008;17:331–7.
[18] Ma WW, Jacene H, Song D, et al. [^{18}F]Fluorodeoxyglucose positron emission tomography correlates with Akt pathway activity but Is not predictive of clinical outcome during mTOR inhibitor therapy. J Clin Oncol 2009;27:2697–704.

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Annals of Oncology Advance Access published October 29, 2011

original article

Annals of Oncology
doi:10.1093/annonc/mdr487

Postprogression survival for first-line chemotherapy of patients with advanced non-small-cell lung cancer

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Received 12 July 2011; revised 5 September 2011; revised 13 September 2011; accepted 14 September 2011

Background: Given the growing number of drugs available for non-small-cell lung cancer (NSCLC), an effect of first-line chemotherapy on overall survival (OS) might be confounded by subsequent therapies. We examined the relation between postprogression survival (PPS) and OS in phase III trials of first-line chemotherapy for advanced NSCLC.
Patients and methods: A literature search identified 69 trials that were published during the past decade. We partitioned OS into progression-free survival (PFS) and PPS and evaluated the relation between OS and either PFS or PPS. We also examined whether any association might be affected by the year of completion of trial enrollment.
Results: The average PPS was longer in recent trials than in older trials (6.5 versus 4.4 months, $P < 0.0001$). For all trials, PPS was strongly associated with OS ($r = 0.82$), whereas PFS was moderately associated with OS ($r = 0.43$). The correlation between OS and PPS in recent trials was stronger than that in older trials ($r = 0.89$ and 0.66).
Conclusions: Our findings indicate that, especially for recent trials, PPS is highly associated with OS in first-line chemotherapy for advanced NSCLC, whereas PFS is only moderately associated with OS.
Key words: chemotherapy, non-small-cell lung cancer, overall survival, phase III trial, progression-free survival

introduction

Lung cancer remains the leading cause of cancer death worldwide [1, 2], with non-small-cell lung cancer (NSCLC) accounting for ~85% of lung cancer cases. Most individuals with NSCLC have metastatic disease at the time of diagnosis and therefore have a poor prognosis. The standard treatment of advanced NSCLC over the past decade has been platinum-based chemotherapy because of the moderate improvement in survival it confers [3–6]. Although many patients initially achieve clinical remission or disease stabilization with first-line chemotherapy, nearly all subsequently experience disease progression and eventually die of advanced NSCLC.

Overall survival (OS) has been traditionally recognized as the most important therapeutic objective for NSCLC patients. However, in view of the growing number of drugs and combinations thereof that are available for the treatment of such patients, any effect of first-line chemotherapy on OS might be confounded by subsequent therapies [7]. Indeed, an improvement in progression-free survival (PFS) has not necessarily resulted in an improved OS in recent randomized trials in patients with NSCLC [8, 9].

The effect of therapies instituted after disease progression on survival in clinical trials is thus of interest. However, little is known about postprogression survival (PPS) in NSCLC. In the present study, we partitioned OS of phase III trials for chemotherapy-naive patients with NSCLC into PFS and PPS and assessed the association of each with OS.

methods

search strategy and selection of trials

An independent review of PubMed citations from 1 January 2000 to 31 October 2010 was carried out. Key words included in the search were 'non–small cell lung cancer', 'clinical trial', 'advanced', and 'chemotherapy'. The search was limited to randomized controlled phase III trials and articles published in English. We reviewed each publication, and phase III studies that compared two or more first-line systemic chemotherapies (including treatment with molecularly targeted agents) for advanced or metastatic NSCLC were selected. To find any additional trials, we searched the reference lists of included trials as well as of large systematic reviews. We also checked articles that were in press at leading journals and searched websites listing abstracts from conferences (organized by the American Society of Clinical Oncology or the Federation of European Cancer Societies). We included trials that provided data for both OS and either PFS or time to progression (TTP), whether or not these parameters were explicitly defined. Trials were excluded if they investigated only immunotherapy regimens or hormonal therapies. Trials that were designed to assess combined modality treatments, including radiation therapy and surgery, were also excluded. To avoid bias, two observers (HH and IO) independently abstracted the data from the trials.

data abstraction

We analyzed in detail the primary and secondary efficacy end points, following the definitions of the authors of each trial. When not specifically

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stated by the authors, we considered the primary end point to be that used for calculation of sample size. For the sake of simplicity, two end points (PFS and TTP) based on tumor assessment are collectively referred to as PFS in the present study, similar to the approach adopted in a recent report [10]. Median OS and median PFS were extracted from all trials that provided data for each treatment group. Median PPS was defined as median OS minus median PFS for each trial. We also obtained the following information from each report: year of completion of trial enrollment, number of patients randomized, number of patients in each treatment arm, number of treatment arms in each trial, proportion of patients who were male or had adenocarcinoma, and median age of the patients.

data analysis

We summarized the survival data (median OS, median PFS, median PPS, and median PFS/median OS) as the average and standard error (SE) for trial arms. SE was calculated on the basis of previously described models [11]. We also calculated the percentage of OS accounted for by PPS for each trial arm as: 100 − (100 × median PFS/median OS). To assess the relation between median OS and either median PFS or median PPS, we used Spearman's rank correlation coefficient. To account for differences in sample size among trial arms, we weighted all analyses by the number of patients in each arm. In addition, all trials were divided into two groups on the basis of the year in which trial enrollment was completed. Given that the median year for completion of enrollment in the 69 analyzed trials was 2002, we dichotomized at year 2002 (older trials, up to and including 2002; recent trials, 2003 and later) in order to evaluate a possible change in PPS, and we assessed whether the evaluated relations might be dependent on the year of completion of trial enrollment. We examined differences in the survival data between older and recent trials by normal approximation of the average survival data (z test). All reported P-values correspond to two-sided tests, and those of P-values <0.05 were considered statistically significant. Analyses were carried out with SAS for Windows release 9.2 (SAS Institute, Cary, NC).

results

characteristics of the trials

Our search yielded a total of 467 potentially relevant publications. Initially, 366 studies were excluded for at least one of the following reasons: they examined other malignancies or combined modality treatments, they were not randomized, they were phase I or II trials, they were review articles, they represented subgroup analyses, or they were duplicates. The selection process for the randomized controlled trials is shown in Figure 1. Review of the remaining 101 publications yielded 69 trials that were considered to be highly relevant for the present study. The main characteristics of the 69 phase III trials included in the analysis are listed in Table 1. A total of 37 986 patients with advanced NSCLC were enrolled, with a median number of patients per study of 433 (range 153–1725). Most of the trials had a high proportion of male patients and of patients with adenocarcinoma. The average median age of the patients was 62.3 years. Ten trials used an end point based on tumor assessment (PFS or TTP) as the primary end point, whereas OS was assessed as the primary end point in 53 trials. The other six trials used response rate or quality of life as the primary end point.

median OS, PFS, and PPS in all trials and in subgroups based on year of completion of trial enrollment

The survival data for trial arms according to the year in which trial enrollment was completed are shown in Table 2. Although the average median PFS in older (up to and including 2002) trials was the same (4.9 months) as that in recent (2003 and later) trials, the average median PPS was ~50% longer in the

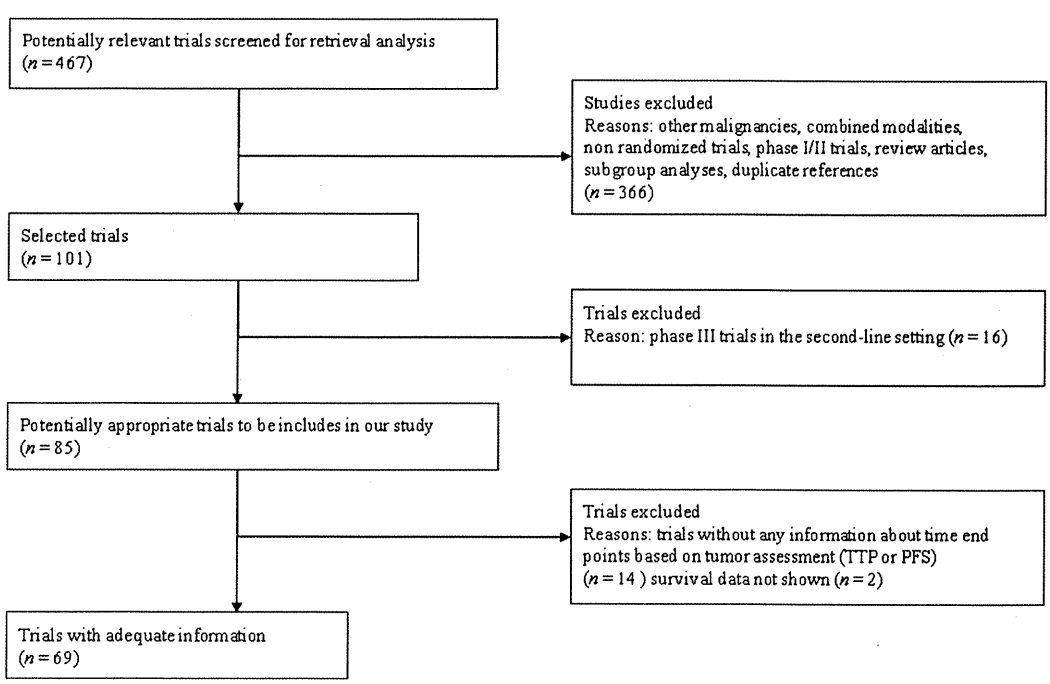

Figure 1. Flow chart showing the progress of trials through the selection process.