Pathol. Oncol. Res.
DOI 10.1007/s12253-011-9465-9

RESEARCH

MUC1 Expression in Pulmonary Metastatic Tumors: A Comparison of Primary Lung Cancer

Kyoichi Kaira · Takehiro Okumura · Kazuo Nakagawa · Yasuhisa Ohde · Toshiaki Takahashi · Haruyasu Murakami · Tateaki Naito · Masahiro Endo · Haruhiko Kondo · Takashi Nakajima · Nobuyuki Yamamoto

Received: 10 May 2011 / Accepted: 19 September 2011

Abstract MUC1 expression has been described as a predictor for tumor progression and worsening of prognosis in various human neoplasms. However, little is known about the role of MUC1 expression in pulmonary metastatic tumors. The aim of this study is to examine the clinicopathological significance of MUC1 expression in pulmonary metastatic tumors (PMT). One hundred forty-seven patients with PMT who underwent ^{18}F-FDG PET before metastasectomy were included in this study. Tumor sections were stained by immunohistochemistry for MUC1, glucose transporter 1 (Glut1), hypoxia-inducible-1α (HIF-1α) and vascular endothelial growth factor (VEGF). ^{18}F-FDG uptake and the expression of these biomarkers were correlated in primary lung cancer. MUC1 expression pattern was classified into high-grade polarized expression (HP), low-grade polarized expression (LP), or depolarized expression (DP) group. Of 147 patients, HP, LP and DP group were 9 (6%), 114 (78%) and 24 (16%), respectively. The expression of Glut1, HIF-1αand VEGF, and ^{18}F-FDG uptake were significantly higher in DP group than HP or LP groups. MUC1 expression with HP and DP pattern was significantly higher in primary lung cancer than in PMT, whereas, MUC1 expression with LP pattern yielded a significantly high positive rate in PMT. LP group was recognized in the majority of patients with pulmonary metastatic adenocarcinoma, especially colon cancer, whereas, HP group was significantly low in pulmonary metastatic adenocarcinoma as compared with primary adenocarcinoma. Polarized MUC1 has a different expression pattern between primary and metastatic tumors with adenocarcinoma, and depolarized MUC1 is closely associated with glucose metabolism and hypoxia.

Keywords MUC1 · Pulmonary metastatic tumor · NSCLC · ^{18}F-FDG PET · Glut1 · Hypoxia

K. Kaira (✉) · T. Takahashi · H. Murakami · T. Naito · N. Yamamoto
Division of Thoracic Oncology, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho Sunto-gun, Shizuoka 411-8777, Japan
e-mail: kkaira1970@yahoo.co.jp

T. Okumura · K. Nakagawa · Y. Ohde · H. Kondo
Division of Thoracic Surgery, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho Sunto-gun, Shizuoka 411-8777, Japan

M. Endo
Division of Diagnostic Radiology, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho Sunto-gun, Shizuoka 411-8777, Japan

T. Nakajima
Division of Pathology, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho Sunto-gun, Shizuoka 411-8777, Japan

Introduction

The impact of a strong expression of MUC1 mucin in various human neoplasms was repeatedly described as a predictor for tumor progression and worsening of prognosis [1–12]. Moreover, MUC1 has emerged as a target molecule in immunotherapy for various cancers [13]. As the mechanism of a target for cancer treatment, unmasked epitopes of MUC1 core protein expressed on tumor cells have been described to be able to elicit a strong antitumor immunity. But, the functional role of MUC1 expression is only partially elucidated.

Lung is one of the major metastatic sites of the neoplasm arising from other organs. Since it is sometimes difficult to

Published online: 02 October 2011

differentiate metastatic pulmonary nodule from primary lung cancer, pulmonary metastastasectomy has become an integral part of diagnosis and treatment if the primary malignancies outside the thorax are controlled. As pulmonary metastatic tumor (PMT) is a heterogenous group of tumors, there is only limited data about the comparison of molecular biology between pulmonary metastatic tumors and primary lung cancer.

Recently, several reports have documented that the over-expression of MUC1 has a crucial role on the cancer progression and metastasis, leading to poor outcome, in patients with non-small cell lung cancer (NSCLC) [3, 4, 13–16]. However, the precise expression profiles of MUC1 have not been yet determinate in PMT. Little is known about how the expression of MUC1 differs between primary lung cancer and PMT.

The usefulness of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) can help predicting the therapeutic response and outcome in PMT patients [17]. The amount of ^{18}F-FDG uptake within tumor cells has been also documented to be determined by the presence of glucose metabolism [glucose transporter 1 (Glut1)], hypoxia [hypoxia-inducible factor-1α (HIF-1α)], and angiogenesis [vascular endothelial growth factor (VEGF)] [17, 18]. Recent experimental studies demonstrated that hypoxia enhances the expression of MUC1 through the direct regulation by HIF-1α in human cancer cell lines [19, 20]. Glut1 and VEGF could be regulated by HIF-1α-dependent way [17, 18], therefore, ^{18}F-FDG PET may be useful to evaluate whether hypoxia is associated with MUC1 expression in human neoplasm.

To elucidate the role of MUC1 expression in PMT, we conducted an immunohistochemical examination of MUC1 in patients with PMT, which was compared with primary lung cancer. In addition, MUC1 expression was correlated with Glut1, HIF-1α, VEGF, and ^{18}F-FDG uptake within tumor cells.

Material and Methods

Patients

We analyzed 170 consecutive patients who underwent ^{18}F-FDG PET and lung resection for pulmonary metastasis from extrathoracic malignancies at Shizuoka Cancer Center between April 2003 and May 2009. The patients who underwent PET study prior to pulmonary metastasectomy were included, and the patients with other malignancies and those who received induction chemotherapy or radiation before pulmonary metastasectomy were excluded from this study. Six patients who received induction chemotherapy or radiation therapy were excluded. Specimens of seven patients were not available. Ten patients were excluded from analysis because they did not have ^{18}F-FDG PET within 4 weeks before their pulmonary resection was performed. Thus, a total of 147 patients who underwent pulmonary metastasectomy were analyzed in the study. All patients were imaged on ^{18}F-FDG PET.

As a test group of pulmonary malignancy, we evaluated MUC1 expression and the biomarkers including ^{18}F-FDG PET in patients with NSCLC, as compared with PMT. One hundred thirty-three NSCLC patients were consecutively assigned in the study between October 2002 and May 2004, and ^{18}F-FDG PET was performed as part of the preoperative workup. These patients underwent surgical management, and the primary lesions were surgically resected. Finally, a total of 126 patients (81 men, 45 women) were eligible in the study. These 126 patients have no pulmonary metastatic tumors due to primary malignancies outside the thorax. Histologically, 82 patients had AC, 36 had SQC, and 8 had other histology. Of the total patients, 63, 25 and 38 had stage I, II and III tumors, respectively. The study protocol was approved by the institutional review board.

Immunohistochemical Staining

Immunohistochemical staining was performed according to the procedure described in the previous reports [3, 17, 18]. The following antibodies were used: a rabbit monoclonal antibody against MUC1 (Ma 552; Novocastra; 1:100 dilution); a rabbit polyclonal antibody against GLUT1 (AB15309, Abcam, Tokyo, Japan, 1:200 dilution); a mouse monoclonal antibody against HIF-1α (NB100-123, Novus Biologicals, Inc., Littleton, 1:50 dilution); a monoclonal antibody against VEGF (Immuno-Biological Laboratories Co.,Ltd., Japan, 1:300 dilution).

According to previous report [3], immunohistochemical analysis of MUC1 expression was evaluated. Firstly, staining density of MUC1 expression was classified into positive or negative, and if positive, each tumor cell was further classified according to the expression pattern into polarized or depolarized expression. According to the percentage of tumor cells showing polarized MUC1 expression and that with depolarized MUC1 expression, MUC1 expression was classified into the high-grade polarized (HP), the low-grade polarized (LP), or the depolarized (DP) group. The classification of MUC1 expression status is as follows: (i) HP when positive percentage of tumor cells with polarized MUC1 expression is more than 50% and positive percentage of tumor cells with depolarized MUC1 expression is less than 10%, (ii) LP when positive percentage of tumor cells with polarized MUC1 expression is less than 50% and positive percentage of tumor cells with depolarized MUC1 expression is less than 10%, (iii) DP when positive percentage of tumor cells with depolarized MUC1 expression is more than 10% regardless of positive percentage of with polarized MUC1 expression. According to

the definition, the patient with tumor showing no MUC1 expression was classified into the LP group.

The expression of Glut1 was considered positive if distinct membrane staining was present. Five fields (X400) were analyzed to determine the frequency of the HIF-1α stained nuclei. For Glut1 and HIF-1α, 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 the total of 1,000 neoplastic cells.

^{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 ^{8}F-FDG and then rested for approximately 1 h before undergoing imaging [17, 18]. 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. 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.

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

Patient Characteristics

The median age of the patients was 64 years (range, 16–82 years). Eighty-one patients were men and 66 were women. The tumor size of resected metastatic tumors ranged from 5 to 68 mm (median, 14 mm). Eastern Cooperative Oncology Group (ECOG) performance status (PS) was 0–1 in all patients. Seventy-five (51%) of 147 patients were smokers. Fifty-seven patients received adjuvant chemotherapy after pulmonary metastasectomy. The organ types of the primary site were as follows: 80 colon cancers, 7 breast cancers, 14 head and neck cancers, 12 soft-tissue sarcomas, 19 genital cancers, 12 gastrointestinal cancers and 3 other cancers. Forty (50%) of 80 patients with colon cancers have a primary site of rectum. Of 12 gastrointestinal cancers, 5 patients have esophageal cancer with SQC and 7 patients gastric cancer with AC. Of 12 sarcomas, 7 patients have osteosarcoma, 3 patients synovial sarcoma and 2 patients malignant fibrous histiocytoma. In NSCLC group, the median size of the resected lesions was 23 mm (range, 6 to 100 mm).

Immunohistochemical Analysis and ^{18}F-FDG PET Findings

Each protein revealed a profile pattern of the unique expression. The immunohistochemical staining was evaluated for the surgically resected 147 pulmonary metastatic lesions. Figure 1 represents the immunohistochemical staining of MUC1 expression. Of all 147 patients, HP, LP and DP group were 9 (6%), 114 (78%) and 24 (16%), respectively. The frequency of LP group was significantly higher than that of HP and LP groups (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 70%. A positive expression of HIF-1α was predominantly expressed in the cytoplasm with some nuclear staining, and was recognized in 70%. The staining pattern of VEGF was uniformly localized in the cytoplasm and/or membrane. The median rate of VEGF positivity was 22.0% (range, 2–76%), and the value of 22% was chosen as a cutoff point. High expression was recognized in 50%.

The mean values (mean and standard deviation) of T/M ratio in PMT and NSCLC were 3.25±0.22 (range, 0.95 to 9.43) and 5.96±0.38 (range, 0.8 to 24.0), respectively. The T/M ratio of PMT was significantly lower than that of NSCLC (p<0.0001). Of patients with NSCLC, The mean values of T/M ratio in AC and SQC were 4.93±0.51 (range, 0.8 to 21.5) and 7.32±0.73 (range, 2.4 to 24.0), respectively, demonstrating statistically significant difference. The T/M ratio of PMT was significantly lower than that of primary lung SQC, but was not than primary lung AC. The median value of T/M ratio in PMT was 3.0, and a median value of 3.0 was used as the cutoff T/M ratio in following analyses. The T/M ratio of more than 3.0 was defined as high expression.

Figure 2 shows the expression of these biomarkers and T/M ratio of ^{18}F-FDG uptake according to MUC1 expression. In PMT patients, the mean scoring of Glut1 and HIF-1α,

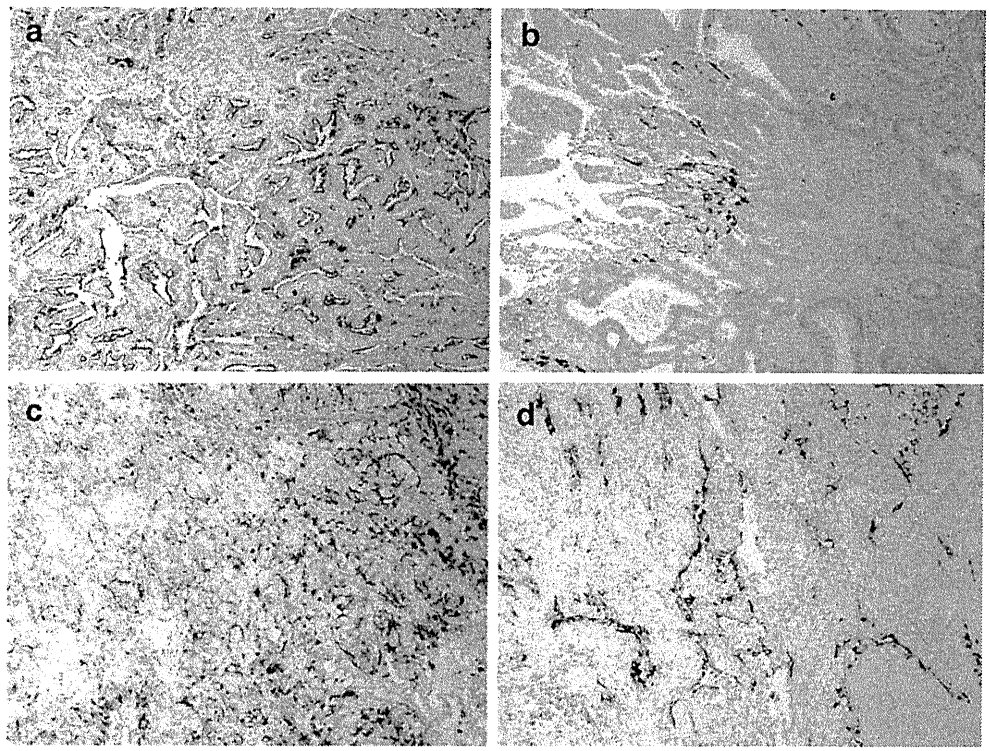

Fig. 1 Immunohistochemical staining of MUC1 expression in pulmonary metastatic tumors: **a** High-grade polarized expression (HP) pattern of MUC1 expression in breast cancer. **b** Low-grade polarized expression (LP) pattern of MUC1 expression in colon cancer. **c** Depolarized expression (DP) pattern of MUC1 in renal cell carcinoma. Immunohistochemical staining of MUC1 expression in primary lung cancer: **d** High-grade polarized expression (HP) pattern of MUC1 expression in pulmonary adenocarcinoma

VEGF positivity, and T/M ratio of ^{18}F-FDG uptake were significantly higher in DP group than HP or LP groups, demonstrating no significant difference between HP and LP groups (Fig. 2a). In patients with primary lung AC, the mean scoring of Glut1 and HIF-1α, VEGF positivity, and T/M ratio of ^{18}F-FDG uptake were significantly higher in LP group than DP group (Fig. 2b). No statistically significant difference in the uptake of ^{18}F-FDG and the meaning scoring of Glut1 and VEGF was observed between HP and LP groups, but uptake of ^{18}F-FDG, the mean scoring of Glut1 and VEGF positivity yielded a statistically significant difference between HP and DP groups. In patients with primary lung SQC, no statistically significant difference in these biomarkers was recognized between HP and LP, between LP and HP, and between HP and DP (Fig. 2c)

Relationship Between MUC1 Expression and Different Variables

The demographic result of the patients according to MUC1 expression is listed in Table 1. The frequency of young age, multiple metastases, large tumor size and a positive Glut1 expression was significantly higher in DP group than in HP group. A statistically significant difference in the age was observed between HP and LP group. The frequency of positive Glut1, HIF-1α and VEGF expression was significantly higher in DP group than in LP group.

We analyzed the expression of MUC1 according to histological types in PMT (Fig. 3a). One hundred and one patients had AC, 15 patients had SQC and 20 patients had sarcoma. In HP group, no significant difference in the positive rate of MUC1 expression was observed among AC, SQC and sarcoma patients. The positive rate of MUC1 expression with LP pattern was significantly higher in AC patients than in SQC patients. But, the positive rate with DP pattern was significantly lower in AC patients than in SQC patients.

According to the organ of the primary sites, the positive rate of MUC1 expression was examined (Fig. 3b). In colon cancer and soft-tissue sarcoma, the positive rate of MUC1 expression with LP pattern was significantly higher than that with HP or DP pattern. In head and neck cancer, MUC1 expression was significantly higher in LP pattern than in HP pattern. In genital cancer, MUC1 expression was significantly higher in DP pattern than HP pattern.

Next, we compared the expression of MUC1 between NSCLC and PMT (Fig. 3). In the analysis of total patients, the MUC1 expression with HP and DP pattern was significantly higher in NSCLC than in PMT, whereas, the MUC1 expression with LP pattern in PMT yielded a significantly high positive rate as compared with NSCLC (Fig. 3c). In AC patients, MUC1 expression with LP pattern was significantly higher in PMT than in NSCLC, whereas, the MUC1 expression with HP pattern in NSCLC yielded a significantly high positive rate as compared with PMT (Fig. 3d). In SQC patients, no significant difference in the positive rate of MUC1 expression was observed between NSCLC and PMT (Fig. 3e).

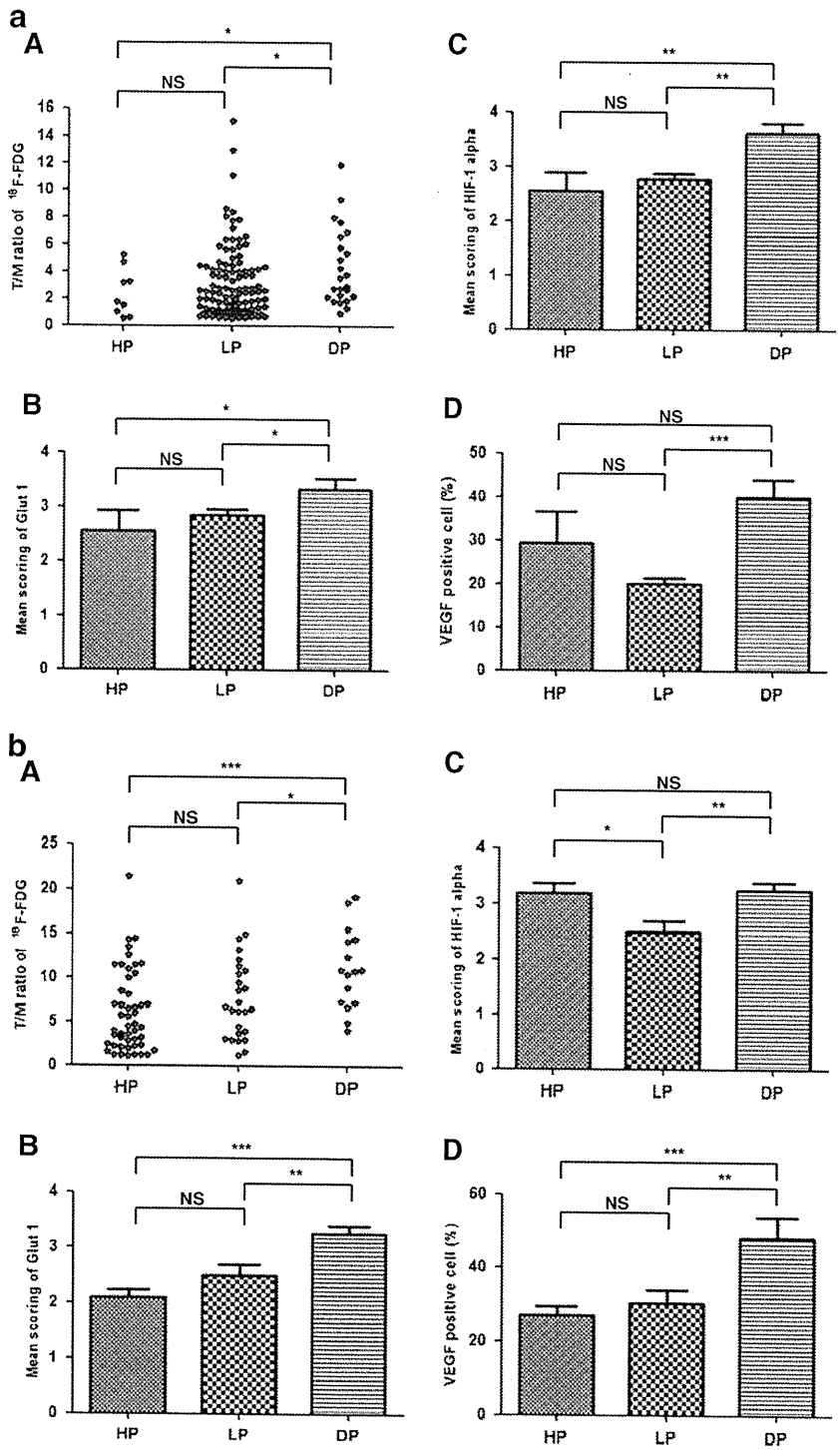

Fig. 2 Comparison of ^{18}F-FDG uptake and angiogenic markers according to MUC1 expression: HP, high-grade polarized expression; LP, low-grade polarized expression; DP, depolarized expression. **a** T/M ratio of ^{18}F-FDG uptake, the mean scoring of Glut1 and HIF-1α, and VEGF positivity of patients with pulmonary metastatic tumors according to MUC1 expression pattern. **b** T/M ratio of ^{18}F-FDG uptake, the mean scoring of Glut1 and HIF-1α, and VEGF positivity of patients with primary lung AC according to MUC1 expression pattern. *P* values indicate significance and were calculated using Fisher's exact test. **c** T/M ratio of ^{18}F-FDG uptake, the mean scoring of Glut1 and HIF-1α, and VEGF positivity of patients with primary lung SQC according to MUC1 expression pattern. *P* values indicate significance and were calculated using Fisher's exact test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. NS, not significant

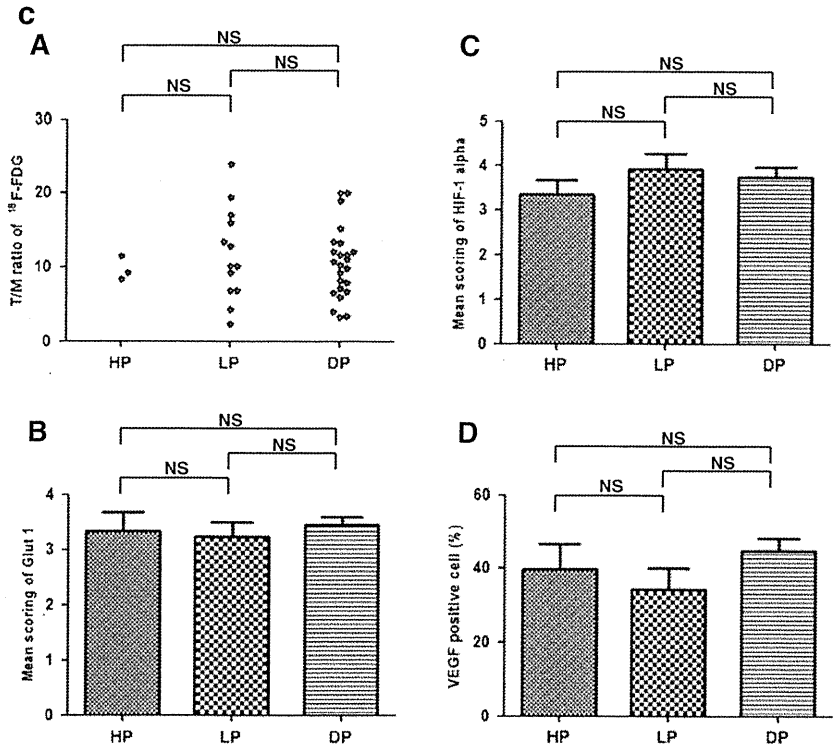

Fig. 2 (continued)

Discussion

This is a clinicopathological study to investigate the expression of MUC1 expression in patients with PMT as compared with NSCLC. MUC1 expression with LP pattern was observed in almost patients with PMT, especially colon cancer and soft-tissue sarcoma. In AC patients, the frequency of LP pattern was significantly higher in PMT tumors than in NSCLC, and MUC1 expression with HP pattern in NSCLC yielded a significantly high positive rate as compared with PMT. A high ^{18}F-FDG uptake in PMT was observed in DP pattern as compared to HP pattern, and the expression of Glut1 and HIF-1α were significantly higher in DP pattern

Table 1 Patient's demographics according to MUC1 expression

Different variables		Total (n=147)	HP (n=9)	LP (n=114)	DP (n=24)	p-value		
						HP/LP	HP/DP	LP/DP
Age	(≤ 65 / > 65 yr)	77 / 70	1 / 8	62 / 52	14 / 10	0.015	0.021	0.822
Gender	(Male / Female)	78 / 69	2 / 7	64 / 50	12 / 12	0.079	0.240	0.654
Smoking	(Yes / No)	76 / 71	2 / 7	63 / 51	11 / 13	0.082	0.263	0.500
PS	(0 / 1)	124 / 23	7 / 2	97 / 17	20 / 4	0.628	1.000	0.762
Tumor size	(≤ 15 / > 15 mm)	63 / 84	7 / 2	48 / 66	8 / 16	0.076	0.046	0.497
No. of meta	(Single / Multiple)	121 / 26	5 / 4	94 / 20	22 / 2	0.071	0.034	0.365
Adjuvant CTx	(Yes / No)	63 / 84	3 / 6	55 / 59	5 / 19	0.497	0.651	0.022
T/M ratio	(High / Low)	58 / 89	4 / 5	42 / 72	12 / 12	0.726	1.000	0.255
Glut 1	(Positive / Negative)	106 / 41	5 / 4	79 / 35	22 / 2	0.462	0.034	0.023
HIF-1α	(Positive / Negative)	103 / 44	6 / 3	75 / 39	22 / 2	1.000	0.110	0.012
VEGF	(Positive / Negative)	71 / 76	5 / 4	45 / 69	21 / 3	0.483	0.068	< 0.01

HP high-grade polarized expression; *LP* low-grade polarized expression; *DP* depolarized expression; *PS* performance status; *No. of meta* Number of resected metastases; *Adjuvant CTx* adjuvant chemotherapy; *Glut1* glucose transporter 1; *HIF-1α* hypoxia inducible factor-1 alpha; *VEGF* vascular endothelial growth factor; *HP/LP* statistical comparison of HP and LP; *HP/DP* statistical comparison of HP and DP; *DP/LP* statistical comparison of DP and LP

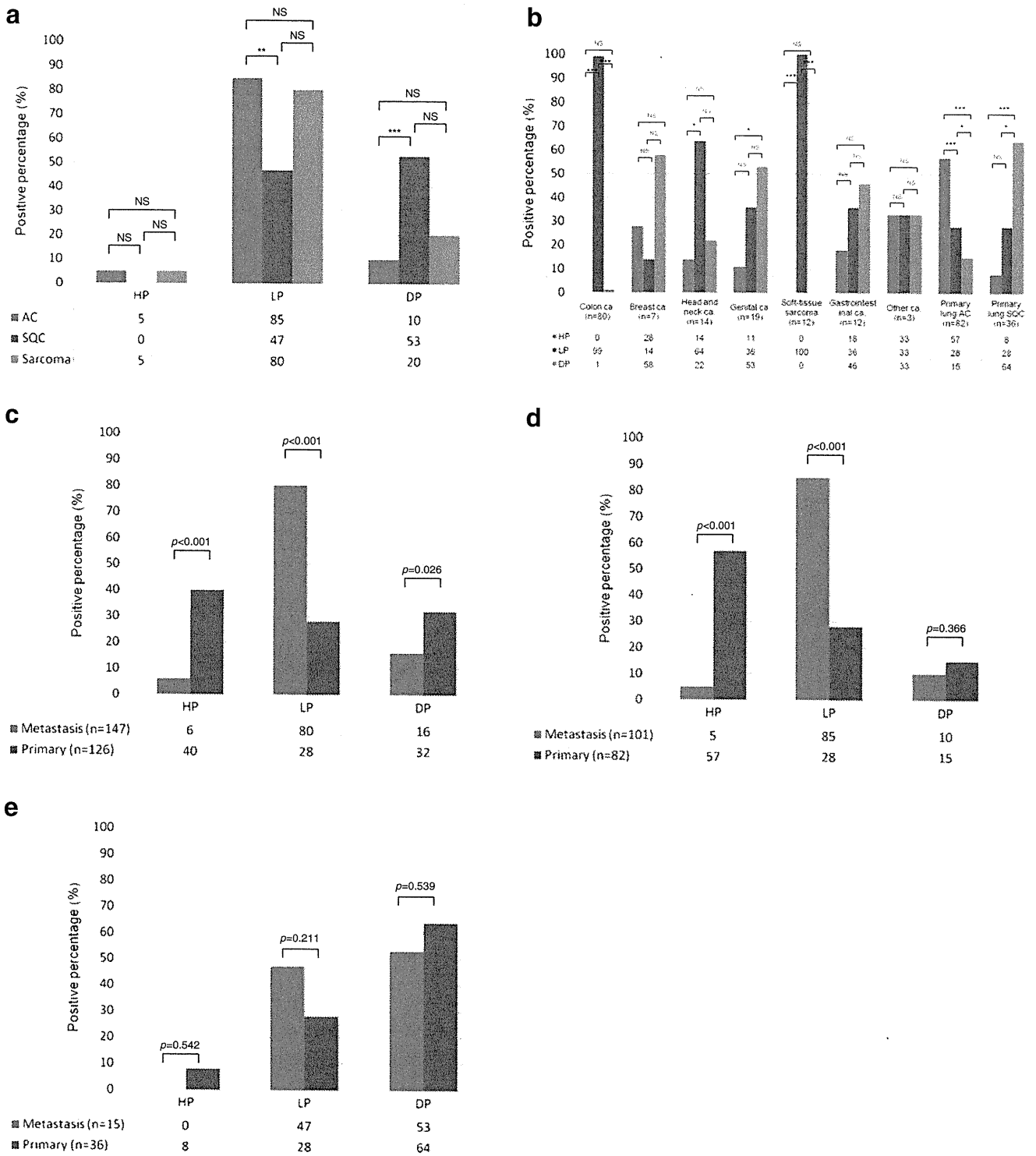

Fig. 3 Comparison of MUC expression according to primary sites and histological types: HP, high-grade polarized expression; LP, low-grade polarized expression; DP, depolarized expression; AC, adenocarcinoma; SQC, squamous cell carcinoma. **a** MUC1 expression according to histological types in pulmonary metastatic tumors. **b** Positive rate of MUC1 expression according to the organ of the primary sites. Comparison of MUC1 expression between primary lung cancer and pulmonary metastatic tumors in total patients (**c**), AC patients (**d**) and SQC patients (**e**). *P* values indicate significance and were calculated using Fisher's exact test. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. NS, not significant

than in HP pattern. This was corresponding to the results of pulmonary lung AC.

MUC1 is a transmembrane mucin consisting of a heavily *O*-glycosylated excellilar domain, a transmembrane domain

and a cytoplasmic tail of 72 amino acids [1]. Recently, several reports have documented that MUC1 expression is correlated with tumor differentiation and postoperative survival in patients with NSCLC [4, 14–16], and Nagai et al has described that depolarized MUC1 expression was a significant and independent prognostic factor to predict poor postoperative prognosis in patients with pulmonary adenocarcinoma and LP or DP expression was mostly observed in moderately to poorly differentiated adenocarcinoma patients [3]. Nagai et al conducted a more detailed MUC1 status classification (HP, LP and DP) for the immunohistochemical evaluation of MUC1 expression in pulmonary tumors [3]. In previous literatures, the immunohistochemical analyses of MUC1 expression were different among the primary tumors and the studies, and the methods used in the studies also have a different technique [4–12]. To analyze the MUC1 expression of pulmonary tumors, therefore, we selected the expression analysis of MUC1 according to Nagai's study [3]. In this study, we could directly compare the expression of MUC1 between NSCLC and PMT.

In our study, low-grade polarized MUC1 expression was observed in the majority of patients with PMT, especially adenocarcinoma such as colon cancer or soft-tissue sarcoma. On the other hand, the frequency of high-grade polarized MUC1 expression was significantly low in pulmonary metastatic adenocarcinoma as compared with primary adenocarcinoma. Only small number of patients with PMT showed the expression pattern of high-grade polarized MUC1, and depolarized MUC1 expression was mainly observed in patients with SQC or genital cancers. In patients with AC as pulmonary nodules, the primary sites are sometimes difficult to differentiate between primary lung cancer and extrathoracic tumor. However, our results suggest that polarized MUC1 (HP or LP pattern) has a markedly different expression pattern between primary and metastatic pulmonary tumors with a histological type of AC. In patients with SQC as pulmonary nodules, whereas, it is difficult to differentiate NSCLC from PMT, because the expression profile of MUC1 was similar among these groups. In addition, ^{18}F-FDG uptake within tumor cells tended to increase from HP, LP to DP pattern, and the expression of Glut1 and HIF-1α was also significantly higher in DP pattern than in HP or LP pattern. Hypoxia has been documented to enhance MUC1 expression in human cancer cell lines, and the present study suggested that hypoxia and glucose metabolism were closely associated with the expression of depolarized MUC1 as compared with that of polarized MUC1. In clinical practice, ^{18}F-FDG PET may be effective for differentiating between polarized MUC1 and depolarized MUC1 expression tumors. However, ^{18}F-FDG PET was not useful for differentiating between HP and LP pattern of MUC1 expression in PMT patients.

MUC1 core protein may be a useful target molecule for immunotherapy in breast cancer, lung cancer and other malignancies expressing MUC1 [21, 22]. MUC1-targeted immunotherapy may be appropriate for such patients as postoperative adjuvant therapy. However, it remains unclear whether MUC1 expression is associated with postoperative outcome in patients with PMT. If not investigate the relationship between MUC1 expression and prognosis, it seems to be difficult to speculate the possibility of a MUC1-targeted immunotherapy after pulmonary metastasectomy in patients with PMT.

In conclusion, polarized MUC1 (HP or LP pattern) had a markedly different expression pattern between primary and metastatic pulmonary tumors with a histology of AC, and depolarized MUC1 was closely associated with glucose metabolism and hypoxia. In addition, ^{18}F-FDG PET may be effective for differentiating between polarized MUC1 and depolarized MUC1 expression tumors. Further study is warranted for investigating the possibility of a MUC1-targeted immunotherapy as a postoperative adjuvant chemotherapy after pulmonary metastasectomy.

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

References

1. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J et al (1990) Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. J Biol Chem 265:15286–15293
2. Schroeder JA, Masril AA, Adriance MC et al (2004) MUC1 overexpression results in mammary gland tumorigenesis and prolonged alveolar differentiation. Oncogene 23:5739–5747
3. Nagai S, Takenaka K, Sonobe M et al (2006) A novel classification of MUC1 expression is correlated with tumor differentiation and postoperative prognosis in non-small cell lung cancer. J Thorac Oncol 1:46–51
4. Woenckhaus M, Merk J, Stoehr R et al (2008) Prognostic value of FHIT, CTNNB1, and MUC1 expression in non-small cell lung cancer. Hum Pathol 39:126–136
5. Utsunomiya T, Yonezawa S, Sakamoto H et al (1998) Expression of MUC1 and MUC2 mucins in gastric carcinomas: its relationship with the prognosis of the patients. Clin Cancer Res 4:2605–2614
6. Hinoda Y, Ikematsu Y, Horinouchi M et al (2003) Increased expression of MUC1 in advanced pancreatic cancer. J Gastroenterol 38:1162–1166
7. Tamada S, Goto M, Nomoto M et al (2002) Expression of MUC1 and MUC2 mucins in extrahepatic bile duct carcinomas: its relationship with tumor progression and prognosis. Pathol Int 52:713–723
8. Kawamoto T, Shoda J, Miyahara N et al (2004) Expression of MUC1 recognized by a monoclonal antibody MY. 1E12 is a useful biomarker for tumor aggressiveness of carcinoma of the gallbladder. Clin Exp Metastasis 21:353–362
9. Feng H, Ghazizadeh M, Konishi H et al (2002) Expression of MUC1 and MUC2 mucin gene products in human ovarian carcinomas. Jpn J Clin Oncol 32:525–529

10. Sivridis E, Giatromanolaki A, Koukourakis MI et al (2002) Patterns of episialin/MUC1 expression in endometrial carcinomas and prognostic relevance. Histopathology 40:92–100
11. Baldus SE, Monig SP, Huxel S et al (2004) MUC1 and nuclear beta-catenin are coexpressed at the invasion front of colorectal carcinomas and are both correlated with tumor prognosis. Clin Cancer Res 10:2790–2796
12. Kirschenbaum A, Itzkowitz SH, Wang JP et al (1999) MUC1 expression in prostate carcinoma: correlation with grade and stage. Mol Urol 3:163–168
13. Broadbent R, Thynne G, McKenzie IFC (1997) Antibody and T cell responses of patients with adenocarcinoma immunized with mannan-MUC1 fusion protein. J Clin Invest 100:2783–2792
14. Hirasawa Y, Kohno N, Yokoyama A et al (2000) Natural antoantobody to MUC1 is a prognostic indicator for non-small cell lung cancer. Am J Respir Crit Care Med 16:589–594
15. Giatromanolaki A, Koukourakis MI, Sivridis E et al (2000) Coexpression of MUC1 glycoprotein with multiple angiogenic factors in non-small cell lung cancer suggests coactivation of angiogenic and migration pathways. Clin Cancer Res 6:1917–1921
16. Situ D, Wang J, Ma Y, et al (2010) Expression and prognostic relevance of MUC1 in stage IB non-small cell lung cancer. Med Oncol Nov 30 [Epub ahead of print]
17. Kaira K, Okumura T, Ohde Y et al (2011) Correlation between ^{18}F-FDG uptake on PET and molecular biology in metastatic pulmonary tumors. J Nucl Med 52:705–711
18. Kaira K, Endo M, Abe M et al (2010) Biologic correlation of 2-[^{18}F]-fluoro-2-deoxy-D-glucose uptake on positron emission tomography in thymic epithelial tumors. J Clin Oncol 28:3746–3753
19. Mikami Y, Hisatsune A, Tashiro T et al (2009) Hypoxia enhances MUC1 expression in a lung adenocarcinoma cell line. Biochem Biophys Res Commun 379:1060–1065
20. Aubert S, Fauquette V, Hémon B et al (2009) MUC1, a new hypoxia inducible factor target gene, is an actor in clear renal cell carcinoma tumor progression. Cancer Res 69:5707–5715
21. Kontani K, Taguchi O, Ozaki Y et al (2003) Dendritic cell vaccine immunotherapy of cancer targeting MUC1 mucins. Int J Mol Med 12:493–502
22. Kufe DW (2009) Functional targeting of MUC1 oncogene in human cancers. Cancer Biol Ther 8:1197–1203

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2011;41(10)1233–1237
doi:10.1093/jjco/hyr119
Advance Access Publication 16 September 2011

Short Communication

Serum Brain-derived Neurotrophic Factor and Antidepressant-naive Major Depression After Lung Cancer Diagnosis

Makoto Kobayakawa[1,2], Masatoshi Inagaki[3,4], Maiko Fujimori[1], Kei Hamazaki[5], Tomohito Hamazaki[5], Tatsuo Akechi[1,6], Shoichiro Tsugane[7], Yutaka Nishiwaki[8], Koichi Goto[8], Kenji Hashimoto[9], Shigeto Yamawaki[2] and Yosuke Uchitomi[1,10,*]

[1]Psycho-Oncology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa-City, Chiba, [2]Psychiatry Division, Graduate School of Biomedical Science, Hiroshima University, Hiroshima-City, Hiroshima, [3]Center for Suicide Prevention, National Institute of Mental Health, National Institute of Neurology and Psychiatry, [4]Department of Psychogeriatrics, National Institute of Mental Health, National Institute of Neurology and Psychiatry, Kodaira-City, Tokyo, [5]Division of Clinical Application, Department of Clinical Sciences, Institute of Natural Medicine, University of Toyama, Toyama-City, Toyama, [6]Department of Psychiatry and Cognitive-Behavioral Medicine, Nagoya City University Graduate School of Medical Science, Nagoya-City, Aichi, [7]Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Chuo-ku, Tokyo, [8]Thoracic Oncology Division, National Cancer Center Hospital East, Kashiwa-City, [9]Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba and [10]Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama-City, Okayama, Japan

*For reprints and all correspondence: Yosuke Uchitomi, Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, 2-5-1 Shikata-cho, Kita-ku, Okayama-City, Okayama 700-8556, Japan. E-mail: uchitomi@md.okayama-u.ac.jp

Received March 1, 2011; accepted July 23, 2011

Previous studies have reported the existence of an association between brain-derived neurotrophic factor and major depression. However, the possible role of brain-derived neurotrophic factor in the pathophysiology of major depression after cancer diagnosis has not yet been investigated. Subjects were collected using the Lung Cancer Database project. Using the cut-off scores on the depression subscale of the Hospital Anxiety and Depression Scale (HADS-D), 81 subjects with depression (HADS-D > 10) and 81 subjects without depression (HADS-D < 5) were selected. The two groups were matched for age, sex, clinical stage and performance status. The serum brain-derived neurotrophic factor levels were measured using an enzyme-linked immunosorbent assay method. The serum brain-derived neurotrophic factor levels were not statistically different between the subjects in the depression group [29.1 (13.6) ng/ml; mean (SD)] and the non-depression group [31.4 (10.6) ng/ml] ($P = 0.22$). In a stratified analysis by gender, however, the mean serum brain-derived neurotrophic factor level in the depression group tended to be lower than that in the non-depression group among women ($n = 24$ pairs, $P = 0.06$). Major depression after cancer diagnosis is not associated with serum brain-derived neurotrophic factor levels.

Key words: major depression – BDNF – lung cancer – cancer diagnosis – stressful event

INTRODUCTION

Cancer is a common and worldwide fatal disease. Learning about the diagnosis of cancer is an extremely stressful life event, and major depression is common among patients with cancer (1). Stressful events are usually considered as strong risk factors for major depression (2). Therefore, the high

prevalence of major depression among cancer patients may be attributable to cancer-specific stressful events (3). However, the pathway by which stressful events lead to major depression among cancer patients has not yet been elucidated.

Recently, brain-derived neurotrophic factor (BDNF) has been recognized as an important factor in the pathophysiology of stress-related mental disorders, particularly major depression (4). In animal studies, the relationships between the stress and decreased expression of BDNF mRNA in the hippocampus and neocortex of rats (5,6), and increased synthesis of BDNF induced by interventions like depression treatments (7,8) were suggested. Patients with major depression had lower levels of serum BDNF than healthy controls (9–11), and the levels of serum BDNF changed to be normal after treatment for depression (9,11). However, with no such studies in the oncologic setting, we preliminarily planed to examine the difference in serum BDNF levels between subjects with and without antidepressant-naive major depression after being diagnosed as having lung cancer, which is a stressful life event and was not considered in the previous human studies (9–11). We hypothesized that the serum BDNF levels in the subjects developing major depression after being diagnosed as having lung cancer would be lower than in those without depression. We secondarily performed a stratified analysis by gender, because a previous study showed significantly low serum BDNF levels in depressive women, but not in depressive men (11).

PATIENTS AND METHODS

Study Design and Subjects

The present study used secondary samples from our previous study (12) on the Lung Cancer Database project (13). The project was a prospective cohort study to investigate the pathogenesis of and the development of new therapy for lung cancer. The project and the present study were approved by the Institutional Review Board and the Ethics Committee of the National Cancer Center, Japan. All participants provided their written informed consent prior to enrollment.

The details of the inclusion and exclusion criteria of the present study were described in our previous report (12). In concise, patients newly diagnosed as having primary lung cancer were included, and patients with cognitive impairment, past or current histories of mental disorders, and brain neoplasms or brain metastasis were excluded. To remove the influence of severe physical status, patients with a performance status (PS) of 2 or higher were also excluded (PS was defined by Eastern Cooperative Oncology Group).

Assessment of Depression

Self-reported questionnaires, including the Hospital Anxiety and Depression Scale (HADS) (14), were completed during the waiting period prior to admission. The HADS consists of seven-item anxiety and seven-item depression subscales and is used to assess anxiety and depressive symptoms during the preceding week. The Japanese version of the depression subscale of the HADS (HADS-D) has two cut-off points that yield a good sensitivity and specificity for depression screening (10 out of 11; 82.4 and 95.1%, major depression only, 4 out of 5; 91.5 and 58.0%, adjustment disorder and major depression, respectively) (15). In this study, 'depression' was defined based on HADS-D scores without usual procedure such as the Structured Clinical Interview for DSM-IV.

Selection of Depression and Non-depression Groups

Subjects were selected according to the method used in our previous study (12), as follows: (i) all eligible subjects were classified into three groups according to the two cut-off points (10 out of 11 and 4 out of 5) for HADS-D; (ii) the number of subjects in the high-score group (>10) was used as the number of cases with major depression; (iii) the same number of controls in the low-score group (<5) was selected from the eligible subjects so that the two groups were matched for age, sex, PS (0 or 1) and clinical stage as assessed by the TNM classification (Ia–IIIa or IIIb–IV). To compare major depression with non-depression, the cases with high HADS-D scores (>10) were enrolled in the 'depression group', and the cases with low scores (<5) were included in the 'non-depression group'.

Measurement of Serum BDNF

Following an overnight fast, blood samples were collected by registered nurses in the morning (7–9 AM), a few days after admission. After storing the samples for about 2 h at 4°C, the serum was separated by centrifugation (1870*g*, 10 min) and stored at −80°C until further assay. The samples were thawed to 4°C and the serum BDNF levels were measured using an enzyme-linked immunosorbent assay kit (Promega, Madison, WI, USA) (9). The absorbance of samples at 450 nm was measured using an Emax automated microplate reader (Molecular Device, Tokyo, Japan).

Assessment of Demographical and Medical Backgrounds

Information regarding clinical, demographic and social factors were collected from the database and the patients' medical charts (13). These data consisted of sex, age, clinical staging as assessed by the TNM classification, PS, pathologic type of the lung cancer, educational level (longer/not longer than 9 years), smoking status, alcohol consumption status, presence/absence of breathlessness and pain, number of platelets and body mass index.

Statistical Analysis

To analyze the background factors, differences in continuous or categorical variables were analyzed by analysis of variance (ANOVA) and the χ^2 test, respectively.

As the primary analysis, the serum BDNF level was analyzed by ANOVA and analysis of covariance (ANCOVA). Background variables that were statistically significantly different between the two groups were examined as independent variables, with the serum BDNF level as the dependent variable, using the Spearman rank correlation coefficient (for continuous variables) or ANOVA (for categorical variables). Only factors that were related to both the background and the BDNF levels were used as covariates in the ANCOVA. As a secondary analysis, stratified analyses according to sex were also performed. All tests were two-tailed, with P values <0.05 indicating statistical significance. The statistical analyses were performed using the statistical software package SPSS for Windows (Version 16.0J, SPSS Japan Institute Inc.)

RESULTS

Participants

During the period of the study, 30 patients refused to participate, while 829 patients provided blood samples and completed self-reported questionnaires. Based on the inclusion/exclusion criteria, 717 patients were found to be eligible for enrollment in the present study (13). Of the 717 subjects, 81 had high HADS-D scores (>10) and were selected as the subjects of the depression group. Of the remaining 319 subjects with HADS-D scores of 4 or under, 81 subjects matched for age and sex were enrolled as controls in the non-depression group.

Group Backgrounds

Table 1 shows the background characteristics of the two groups, including some data that were reported in our previous study (12). The depression group contained more subjects with breathlessness than the non-depression group. Except for the breathlessness, no other variable differed significantly between the groups. The mean and standard deviation in the interval between completion of the HADS questionnaire and the blood sampling in all the subjects were 3.6 and 5.0 days, respectively; these values were similar for both groups [depression group; 3.9 (5.0) days; mean (SD), non-depression group; 3.8 (5.9) days] ($F = 0.04$, $P = 0.85$). The serum BDNF levels showed no significant differences between the subjects with breathlessness [$n = 82$; 28.7 (11.3) ng/ml; mean (SD)] and those without breathlessness [$n = 78$; 31.9 (13.0) ng/ml] ($F = 2.66$, $P = 0.11$).

Table 1. Background of all subjects ($n = 162$)

	Depression	Non-depression	χ^2 or F^a	P-value
HADS-D (score)	11–21	0–4		
Number	81	81		
Sex (male)	57 (70%)	57 (70%)	0.0	1.00
Age (y.o.)	65.1 ± 8.3	65.0 ± 8.3	0.003[a]	0.96
Performance Status (0/1)[b]	23/58	23/58	0.0	1.00
Clinical stage				
Ia–IIIa[c]	34 (42%)	34 (42%)	0.0	1.00
IIIb–IV[c]	47 (58%)	47 (58%)		
Educational level (>9 years)	52 (64%)	56 (69%)	0.46	0.50
Alcohol (>45 g/day)	14 (17%)	12 (15%)	0.38	0.54
Current smoker	33 (41%)	30 (37%)	0.23	0.63
Pathology				
Adenocarcinoma	42	45	1.49	0.83
Squamous cell	19	20		
Small cell	6	7		
Large cell	8	6		
Other	6	3		
Breathlessness (presence)	49 (60%)	33 (41%)	5.61	0.018
Pain (presence)	28 (35%)	31 (38%)	0.19	0.67
Body mass index (kg/m^2)	22.0 ± 3.5	22.1 ± 3.2	0.06[a]	0.80
Platelet ($10^4 \times \mu l$)	27.7 ± 9.3	28.3 ± 9.4	0.20[a]	0.66

Age, body mass index and platelet: mean ± SD. PS: number. Others: number and percentage.
[a]F-value.
[b]Defined by Eastern Cooperative Oncology Groups.
[c]Defined by TNM Classification, International Union Against Cancer.

Serum BDNF Levels in the Two Groups

Figure 1 illustrates the absence of any significant difference in the serum BDNF levels between the depression group and the non-depression group (ANOVA). The serum BDNF levels were normally distributed. Since no covariates were detected as statistically significant variables in the background analyses, ANCOVA was not performed. In the stratified analyses by gender, no significant differences were seen between the two groups among the men. The mean serum BDNF level was lower in the women with depression than in the women without depression, but the difference was not statistically significant.

DISCUSSION

This is the first study, to the best of our knowledge, conducted to investigate the association between serum BDNF levels and major depression in the oncologic setting.

Unlike in previous studies (9–11), the serum BDNF levels were not lower in the subjects with major depression in the

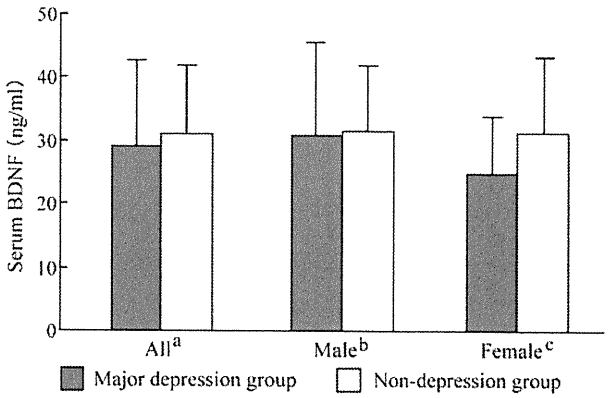

Figure 1. Serum levels of brain-derived neurotrophic factor (BDNF) in the depression group and in the non-depression group. [a]The primary analysis showed the absence of any statistically significant differences in the serum BDNF levels between the subjects in the depression group [$n = 81$; 29.1 (13.6) ng/ml; mean (SD)] and the non-depression group [$n = 81$; 31.4 (10.6) ng/ml] ($F = 1.53$, $P = 0.22$). [b]A stratified analysis by gender showed the absence of any statistically significant difference in the levels between the depression group [$n = 57$; 30.9 (14.8) ng/ml; mean (SD)] and the non-depression group [$n = 57$; 31.8 (10.2) ng/ml] ($F = 0.13$, $P = 0.72$) among men. [c]A stratified analysis by gender also showed the absence of any statistically significant difference between the depression group [$n = 24$; 24.7 (9.1) ng/ml; mean (SD)] and the non-depression group [$n = 24$; 30.7 (11.7) ng/ml] ($F = 3.87$, $P = 0.06$) among women.

present study. The lack of difference in the serum BDNF in our study might be related to the characteristics of depression in oncologic settings, which tends to be reactive to stressful event, mild and of short duration (3,16). In a previous study in which psychiatric patients without cancer were examined, the mean durations of depressive episodes were 0.78 years (9). Of the 81 cancer patients with major depression in the present study, 60 completed the HADS questionnaire within 1 month of the disclosure of their lung cancer diagnosis. None of the subjects in the major depression group visited the clinical psychiatric service or received antidepressants before or after their enrollment in this study. Although the duration of major depression was not directly assessed, the subjects with major depression in the present study might have had mild depression of short duration that remitted by themselves without antidepressants. The associations between peripheral BDNF and severity or duration of depressive episode were not concluded (17). Further study may be needed.

In the present study, depression was defined using the cut-off scores of the HADS-D and not by a structured psychiatric interview (such as the Structured Clinical Interview for DSM-IV). The one-point assessment of HADS-D might not always indicate a major depressive episode defined by DSM-IV; this could be a reason why the present result differ from previous studies' (9–11).

Although the P value did not reach statistical significance, our secondary analysis showed that women with major depression tended to have a lower serum BDNF level than women without depression. This result may support the result of a previous study suggesting an important role of reduced serum BDNF in depressive women, but not in men (11). Other studies reported an association between BDNF and the menstrual cycles in humans (18) and sex hormones in animals (19). Further studies examining these factors may be useful for elucidating the association between BDNF and major depression.

This study had the following limitations: (i) subjects with severe depression might have been excluded from this study because subjects with poor physical activity and cognitive impairment were ineligible and 30 subjects refused to participate in this study. (ii) Although peripheral BDNF was suggested to partly reflect the BDNF levels in cerebral spinal fluids (18,20), serum BDNF was mainly stored in platelets. Relation of serum BDNF levels to BDNF in hippocampus was uncertain. Further studies may be needed to reach definitive conclusions.

Acknowledgements

The authors thank Drs Nobuya Akizuki, Eisho Yoshikawa and Eiji Shimizu.

Funding

This work was supported in part by the Third-Term Comprehensive 10-Year Strategy for Cancer Control and Research, Japanese Ministry of Health, Labour, and Welfare.

Conflict of interest statement

None declared.

References

1. Derogatis LR, Morrow GR, Fetting J, Penman D, Piasetsky S, Schmale AM, et al. The prevalence of psychiatric disorders among cancer patients. *JAMA* 1983;249:751–7.
2. Kendler KS, Karkowski LM, Prescott CA. Causal relationship between stressful life events and the onset of major depression. *Am J Psychiatry* 1999;156:837–41.
3. Chochinov HM. Depression in cancer patients. *Lancet Oncol* 2001;2:499–505.
4. Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. *Arch Gen Psychiatry* 1997;54:597–606.
5. Vaidya VA, Marek GJ, Aghajanian GK, Duman RS. 5-HT2A receptor-mediated regulation of brain-derived neurotrophic factor mRNA in the hippocampus and the neocortex. *J Neurosci* 1997;17:2785–95.
6. Smith MA, Makino S, Kvetnansky R, Post RM. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* 1995;15:1768–77.
7. Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* 1995;15:7539–47.
8. Altar CA, Whitehead RE, Chen R, Wortwein G, Madsen TM. Effects of electroconvulsive seizures and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. *Biol Psychiatry* 2003;54:703–9.

9. Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, et al. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry* 2003;54:70–5.
10. Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry JM, Bertschy G. Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biol Psychiatry* 2005;57:1068–72.
11. Huang T-L, Lee C-T, Liu Y-L. Serum brain-derived neurotrophic factor levels in patients with major depression: effects of antidepressants. *J Psychiatr Res* 2008;42:521–5.
12. Kobayakawa M, Yamawaki S, Hamazaki K, Akechi T, Inagaki M, Uchitomi Y. Levels of omega-3 fatty acid in serum phospholipids and depression in patients with lung cancer. *Br J Cancer* 2005;93:1329–33.
13. Nakaya N, Goto K, Saito-Nakaya K, Inagaki M, Otani T, Akechi T, et al. The Lung Cancer Database Project at the National Cancer Center, Japan: study design, corresponding rate, and profiles of cohort. *Jpn J Clin Ooncol* 2006;36:280–4.
14. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983;67:361–70.
15. Kugaya A, Akechi T, Okuyama T, Okamura H, Uchitomi Y. Screening for psychological distress in Japanese cancer patients. *Jpn J Clin Oncol* 1998;28:333–8.
16. Inagaki M, Matsuoka Y, Sugahara Y, Nakano T, Akechi T, Fujimori M, et al. Hippocampal volume and first major depressive episode after cancer diagnosis in breast cancer survivors. *Am J Psychiatry* 2004;161:2263–70.
17. Molendijk ML, Bus BA, Spinhoven P, Penninx BW, Kenis G, Prickaerts J, et al. Serum levels of brain-derived neurotrophic factor in major depressive disorder: state-trait issues, clinical features and pharmacological treatment. *Mol Psychiatry* advance online publication 21 September 2010; doi:10.1038/mp.2010.98 (in press).
18. Lommatzsch M, Zingler D, Schuhbaeck K, Schloetcke K, Zingler C, Schuff-werner P, et al. The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiol Aging* 2005;26:115–23.
19. Cavus I, Duman RS. Influence of estradiol, stress, and 5-HT2A agonist treatment on brain-derived neurotrophic factor expression in female rats. *Biol Psychiatry* 2003;54:59–69.
20. Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ. Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacology* 1998;37:1553–61.

Phase II Study of Topotecan with Cisplatin in Japanese Patients with Small Cell Lung Cancer

YUKIO HOSOMI[1], MASAHIKO SHIBUYA[1], SEIJI NIHO[2], YUKITO ICHINOSE[3], KATSUYUKI KIURA[4], HIROSHI SAKAI[5], KOJI TAKEDA[6], SHINZO KUDO[7], KENJI EGUCHI[8], KAORU MATSUI[9], NORIYUKI MASUDA[10], MASAHIRO ANDO[11] and KOSHIRO WATANABE[12]

[1]*Division of Respiratory Medicine, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan;*
[2]*Department of Thoracic Oncology, National Cancer Center Hospital East, Chiba, Japan;*
[3]*Department of Thoracic Oncology, National Kyushu Cancer Center, Fukuoka, Japan;*
[4]*Department of Allergy and Respiratory Medicine, Okayama University Hospital, Okayama, Japan;*
[5]*Division of Thoracic Oncology, Saitama Cancer Center, Saitama, Japan;*
[6]*Department of Clinical Oncology, Osaka City General Hospital, Osaka, Japan;*
[7]*Department of Respiratory Medicine, Osaka City University Hospital, Osaka, Japan;*
[8]*Division of Medical Oncology, Tokai University Hospital, Kanagawa, Japan;*
[9]*Department of Thoracic Malignancy, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Osaka, Japan;*
[10]*Department of Respiratory Medicine, Kitasato University Hospital, Kanagawa, Japan;*
[11]*Division of Internal Medicine, Tsuboi Cancer Center Hospital, Fukushima, Japan;*
[12]*Department of Respiratory Medicine, Yokohama Municipal Citizen's Hospital, Kanagawa, Japan*

Abstract. *Background: We conducted a phase II study of topotecan (Tp) with cisplatin (CDDP) in previously untreated Japanese patients with extensive-disease small cell lung cancer (ED-SCLC). Patients and Methods: In stage 1, a total of 30 patients were allocated to Tp 0.65 mg/m^2 with CDDP 60 mg/m^2 day 1 or Tp 1.00 mg/m^2 with CDDP day 5 following prophylactic granulocyte colony stimulating factor (G-CSF) from day 6. In stage 2, the selective combination in 29 patients was evaluated for response rate, toxicity and overall survival. Results: In stage 1, Tp 1.00 mg/m^2 with CDDP day 5 was selected this schedule had a better hematological profile. In stage 2, the response rate was 83%, and grade 3/4 adverse events were hematological-toxicities. The median survival time was 17.5 months and the 1 year survival rate was 79%. Conclusion: Combination of Tp and CDDP on day 5 with G-CSF support is safe and effective for previously untreated ED-SCLC Japanese patients.*

Correspondence to: Yukio Hosomi, 18-22, Honkomagome 3 chome, Bunkyo-ku, Tokyo, 113-8677, Japan. Tel: +81338232101, Fax: +81338241552, e-mail: yhosomi@cick.jp

Key Words: Cisplatin, small cell lung cancer, topotecan.

Most patients with previously untreated small cell lung cancer (SCLC) are highly sensitive to chemotherapy and radiation therapy. As cisplatin (CDDP) is the most important drug for SCLC chemotherapy, the standard chemotherapy regimen for treatment of extensive-disease (ED)-SCLC has been the combination of CDDP plus etoposide (PE regimen). Although this combination has produced objective response rates as high as 80%, median survival times range from 9 to 11 months, with a 2-year survival rate of less than 10% (1-3). Several novel strategies failed to improve patient survival time. Therefore, investigation of therapy resulting in improvement of survival is still ongoing.

Inhibitors of topoisomerase I (Topo I, an enzyme necessary for DNA replication) are active against SCLC. A randomized study of the Topo I inhibitor, irinotecan, plus CDDP *versus* PE in previously untreated Japanese patients with ED-SCLC indicated survival benefit of Topo I inhibitor (4). Topotecan (Tp), a cytotoxic water-soluble semisymthetic camptothecin analogue, acts as an inhibitor of Topo I. Tp demonstrated anti tumor activity towards human cancer cell lines and animal tumor models, then a combination of Tp and CDDP had synergistic effect *in vitro* studies (5, 6). The combination effect of Tp and CDDP is not influenced by the order of administrating the two drugs. On the contrary, adverse effects are influenced (7). Therefore Tp has been evaluated as useful

0250-7005/2011 $2.00+.40

for ovarian and uterine cervical carcinoma, besides second-line therapy in SCLC (8-10). The combination regimen of Tp with CDDP has been documented in use for previously untreated ED-SCLC patients (11, 12). In addition, no difference in efficacy and tolerability was recorded in Tp and CDDP combination regimen compared with standard regimen in a phase III study for the first-line therapy of previously untreated ED-SCLC patients (13, 14).

Although the standard regimen for ED-SCLC in North America and Europe is a combination of CDDP plus etoposide (3), the combination of irinotecan plus CDDP is the standard regimen in Japan due to the significant prolongation of survival obtained from the interim analysis of a phase III clinical study by the Japan Clinical Oncology Group (JCOG) (4), with similar results to the phase II clinical trial (15) on this regimen.

Since there are different antitumor efficacies of Topo I inhibitor between Japanese patients and North American and European populations, the present phase II clinical trial was planned to investigate antitumor effects of Tp plus CDDP in Japanese patients with ED-SCLC, for whom irinotecan plus CDDP is the standard regimen.

The recommended schedule was evaluated at stage I due to the need for estimation of different schedule dependency on efficacy and safety for Japanese patients. Based on findings from two clinical studies on irinotecan plus CDDP (phase II and phase III), the clinical efficacy of the combination of CDDP plus Tp, which has the same Topo I inhibitory activity with irinotecan, is evaluated in this phase II clinical trial to investigate whether the efficacy of irinotecan plus CDDP is based on the mode of action of Topo I inhibitor plus CDDP in Japanese patients.

In a completed phase I study, we determined the recommended dose of Tp 0.65 mg/m^2 and 1.00 mg/m^2 as in combination with CDDP day 1 and day 5 schedules, respectively, with fixed dose of CDDP at 60 mg/m^2 (16). We found novel potential of the Tp combination regimen with CDDP against previously untreated Japanese patients with ED-SCLC in a phase II study. In present study, the dose of CDDP at 60 mg/m^2 was fixed. A prophylactic G-CSF concomitant treatment was employed from consideration of dose limiting factors of this combination. In addition to efficacy and safety evaluation of Tp plus CDDP combination, the treatment effect of the combination regimen of Topo I inhibitor with CDDP in Japanese patients was also evaluated.

Patients and Methods

Study design. A two-stage study was designed (Figure 1). At stage 1, two arms (arm A and arm B) were compared evaluating tumor response and toxicity to select the superior arm. In stage 2, 15 cases were added to the selected arm. The cases in stage 1 and stage 2 of the selected arm were combined for evaluation of efficacy and safety of this combination.

Eligibility. Japanese patients with histological and/or cytological documented SCLC were eligible for this study. Each patient was required to meet the following criteria: ED-SCLC, previously untreated, having measurable lesion; performance status (Eastern Cooperative Oncology Group: ECOG PS) of 0-1; age 20 to 74 years; adequate organ function (hemoglobin level >9.5 g/dl, leukocyte count 4,000 to 12,000/mm^2, neutrophill cell count >2,000/mm^2, platelet count >100,000/mm^2, aspartate aminotransferase (AST) level <2.5 times of the normal upper limit, total bilirubin value <1.5 mg/dl, serum creatinine below the normal upper limit, resting partial pressure oxygen >60 torr; a life expectancy of at least 3 months; hospitalized; and written informed consent obtained. The protocol and informed consent procedures were reviewed and approved by the Institutional Review Board of each participating institute. This study was subjected to Good Clinical Practice (GCP) and Declaration of Helsinki.

Treatment schedule. Tp of 0.65 mg/m^2 for CDDP day 1 schedule (arm A) or 1.00 mg/m^2 (or 1.2 mg/m^2, if the nadir of the first cycle for leucocytes of >2,000/mm^2 and platelet of >50,000/mm^2, the dose from next cycle could be increased) for CDDP day 5 schedule (arm B) were intravenously administered at over 30 min by drop infusion for the first 5 consequent days within one cycle of 21 days. Tp was provided by Nippon Kayaku Co., Ltd. as 1.1 mg/vial formulation to be dissolved in 500 to 1,000ml of saline. CDDP was also intravenously administered at over 2 h by drop infusion at day 1 (arm A) or day 5 (arm B). Prophylactic G-CSF was administered from day 6 (day after final Tp administration) until recovery from nadir for leucocytes of >10,000/mm^2 or neutrocytes of >5,000 mm^2. For each patient, 4 cycles were planned.

Dose modification. When grade 4 neutropenia, more than grade 3 febrile neutropenia with over 38.5°C or thrombocytopenia (<25,000/mm^2) occurred, the Tp dose was reduced from 0.65 to 0.5 mg/m2 for arm A or 1.0 to 0.8 mg/m^2 for CDDP day 5 schedule (or 1.2 to 1.0 mg/m^2 if applicable), respectively. When the leukocyte count >4,000/mm^2, neutrocyte count >2,000/mm^2, platelet count >100,000/mm^2 and hemoglobin value >8,0g/dl or recovery tendency was observed, the treatment was able to proceed to the next cycle. Treatment could be delayed for up to 30 days from day 1 of the current cycle to allow a patient sufficient time to recover from study drug-related toxicity.

Evaluation. All patients underwent weekly evaluations that included assessment of symptoms (subjective and objective findings), a physical examination, a complete blood cell count, blood chemistry (including measurement of AST, alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (Al-P), total bilirubin, total protein, serum creatinine, blood urea nitrogen (BUN), serum electrolytes) and urinalysis. Toxicity was evaluated according to NCI CTCAE version 3 criteria (17). Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) (18), assessed by computed tomographic (CT) scanning, as with staging at enrollment. All the observed responses were reviewed by an extramural panel.

Statistical analysis. This study was made up of two different stages, one for the CDDP schedule selection (stage 1) and the other for the evaluation of the selected schedule (stage 2). The primary aim of this study was to assess the anti-tumor effect of the combination. Thus, involvement of 15 cases for the two CDDP administration

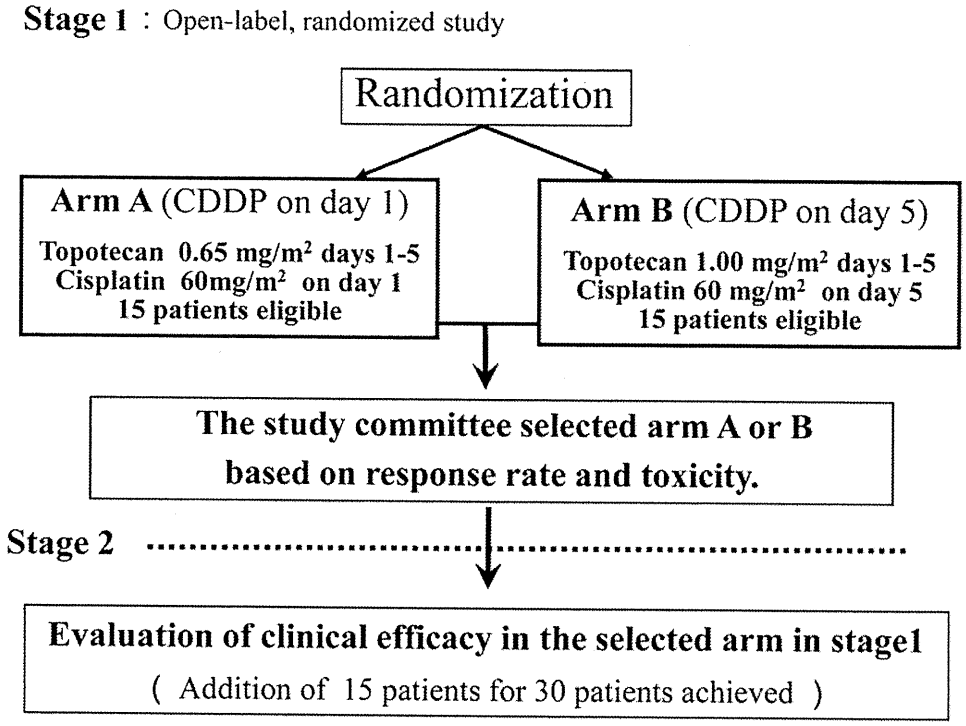

Figure 1. *The study design is shown graphically. Stage 1: comparison phase. Stage 2: evaluation phase.*

schedules (on day 1 and day 5) were adequate based on the following premises: 8 cases of rejection region, 70% expected efficacy rate, 35% threshold response rate, one-sided significance level α=0.042 and power 1-β=0.869.

The anti-tumor activity was estimated by response rate and 95% confidence intervals. Toxicity was estimated by subjective and objective findings and incidence by, in comparison between CDDP day 1 and day 5 schedules. Since the stage 1 study was not statistically powered, the two arms were analyzed separately.

At stage 2, clinically useful threshold and expected efficacy rate were set as 60% and 85% respectively. On this basis, 26 cases were required based on α=0.05 (bilaterally) and β=0.2 (one-sided), thus the target number of cases use 30. In addition to stage 1, 15 cases were planned to be enrolled for the selected superior arm. The primary endpoint of stage 2 was to assess the anti tumor efficacy of Tp in combination with CDDP, taking the toxicity profile into account. The secondary endpoint was overall survival, and the several survival curves were estimated by means of the Kaplan and Meier method.

Results

From August 2005 through July 2008, a total of 44 chemotherapy-naive Japanese ED -SCLC patients were enrolled into this study from 16 institutions. All 44 cases were eligible. In stage 1, 30 patients were randomly assigned to the arms of CDDP day 1 and day 5 schedules. In stage 2, 14 patients were enrolled for arm B, in addition to stage 1 (total 29 cases for this schedule). All enrolled patients were included in analyses of tumor response and toxicity. Survival times of 29 patients for CDDP day 5 schedule were evaluated. Patients' characteristics are listed in Table I. For arm B, 6 female patients were enrolled in stage 1. Median ages for patients in arms A and B in stage 1 were 62 (55 to 74) and 66 (56 to 74) years, respectively. There were no enrolment criteria or protocol violations. Total cycles for each stage are listed in Table II. Median treatment of cycles was 4 in both stage 1 and stage 2.

Response. Clinical response for stage 1 is listed in Table II. Arm A resulted in 12 partial responses (PRs), 1 case of stable disease (SD) and 1 case not evaluated (NE). The response rate was 80% (95% CI of 51.9 to 95.7%). Arm B had 12 PRs, 2 case of SD and 1 case of progressive disease (PD). The response rate of arm B was 80% (95% CI of 51.9 to 95.7%). In stage 1, there was no difference in the response rate between arm A and arm B. It is notable that 1 complete response (CR) was observed in stage 2. Total response for CDDP day 5 schedule (29 cases in stage 1 and 2) was 1 CR, 23 PRs, 3 SDs, and 2 NEs with median response rate of 83% (95% CI of 64.2 to 94.2%). As of November 2009, when the final analysis was conducted, the median overall survival was 17.5 months (95% CI of 14.8 to 20.8 months) for CDDP day 5 schedule. A Kaplan-Meier curve for survival of patients in arm B is indicated in Figure 2. Overall survival rate for this group was 79% (95% CI of 64.6 to 94.1%) at 1 year.

Table I. *Patient characteristics.*

	Stage 1		Stage 2
	CDDP on day 1	CDDP on day 5	CDDP on day 5
No. of patients	15	15	14*
Gender			
Male	15	9	12
Female	0	6	2
Age (years)			
Median	62.0	66.0	64.0
Range	55-74	56-74	46-73
Performance status (ECOG)**			
0	5	4	4
1	10	11	10
Stage			
IIIB	0	3	2
IV	15	12	12
Tumor diameter (mm)			
50-100<	1	2	1
≥100	14	13	13
Metastasis			
Lung	3	3	3
Liver	7	4	2
Brain	6	1	3
Bone	4	5	6

*Additional patients in stage 2. **Eastern cooperative oncology group.

Table II. *Number of treatment cycles and clinical response.*

	Stage 1		Stage 2
	CDDP on day 1	CDDP on day 5	CDDP on day 5
Topotecan (mg/m^2)	0.65	1.00	1.00
No. of patients	15	15	29*
Count of treatment cycles			
Count of total cycles	52	53	110
Median cycle	4	4	4
Clinical response**			
Complete response	0	0	1
Partial response	12	12	23
Stable disease	1	2	3
Progressive disease	1	0	0
Not evaluable	1	1	2
Response rate (%)	80	80	83
95% Confidence interval (%)	51.9-95.7	51.9-95.7	64.2-94.2

*Selected 15 patients of CDDP on day 5 in stage 1 + additional 14 patients in stage 2. **Evaluated according to RECIST.

Toxicity. The toxicity profile in stage 1 is listed in Table III. No death or febrile neutropenia was observed for any of the 30 patients in stage 1. The main grade 3/4 adverse events in stage 1 were hematological toxicities: leukopenia, neutropenia, thrombocytopenia and anemia; were observed as 40%, 67%, 67% and 60% for arm A and 7%, 40%, 60% and 47% for arm B, respectively. Arm A had a tendency for higher incidence of adverse events than arm B. Non-hematological events (subjective and objective findings) in stage 1 were nausea, anorexia, constipation, vomiting, fatigue and alopecia, with a range of 53% to 93%. As Grade 3 events, anorexia, fatigue and body weight loss were observed each for 1 patient. There was no difference in non-hematological toxicity profile between the two CDDP schedules. Over 50% of patients in stage 1 experienced increased AST and/or ALT, with 2 cases of grade 3 AST increase. Because no difference in the efficacy between the two arms was observed, the CDDP day 5 schedule was employed for stage 2, taking the hematological toxicity profile into account with lower incidence of grade 3 events in arm B compared with arm A in stage 1. The toxicity profile of stage 2 is listed in Table III. No death or febrile neutropenia was observed in this group. The main adverse events in stage 2 were similar to those of arm B in stage 1, both in nature and grade.

Discussion

In this study, Tp at 0.65 mg/m^2 and 1.00 mg/m^2 were employed for CDDP day 1 and day 5 schedules, respectively, based on the results of a previous phase I study. In stage 1, CDDP schedules of day 1 and day 5 administration were evaluated to select optimum combination with Tp, taking response and toxic profile into account. In stage 2, additional patients were enrolled onto the superior regimen to evaluate response rate as primary endpoint and overall survival (time and 1-year rate) as secondary endpoints. In stage 1, both arms had similar responses, with different hematological toxicity profiles. In arm A, the incidence of grade 3/4 hematological events were slightly higher than those in arm B. From the safety point of view, the CDDP day 5 schedule was employed for further study.

Although the primary endpoint of stage 1 was the response rate of this combination by schedule, there were no substantial difference between the two schedules, hence the toxicity profile was taken into account for the selection of the superior arm.

In a previous report from North America/Europe, death from sepsis with the CDDP day 1 regimen was shown, indicating the possibility of severe hematological toxicity (19), and then the validity of CDDP day 5 schedule was suggested (7, 12, 19). Although the administration timing of CDDP in the combination is the same for Japanese patients, in arm A in this study, no death or febrile neutropenia was observed, likely due to the contribution of G-CSF from day 6. The response rate for the 29 cases of CDDP day 5

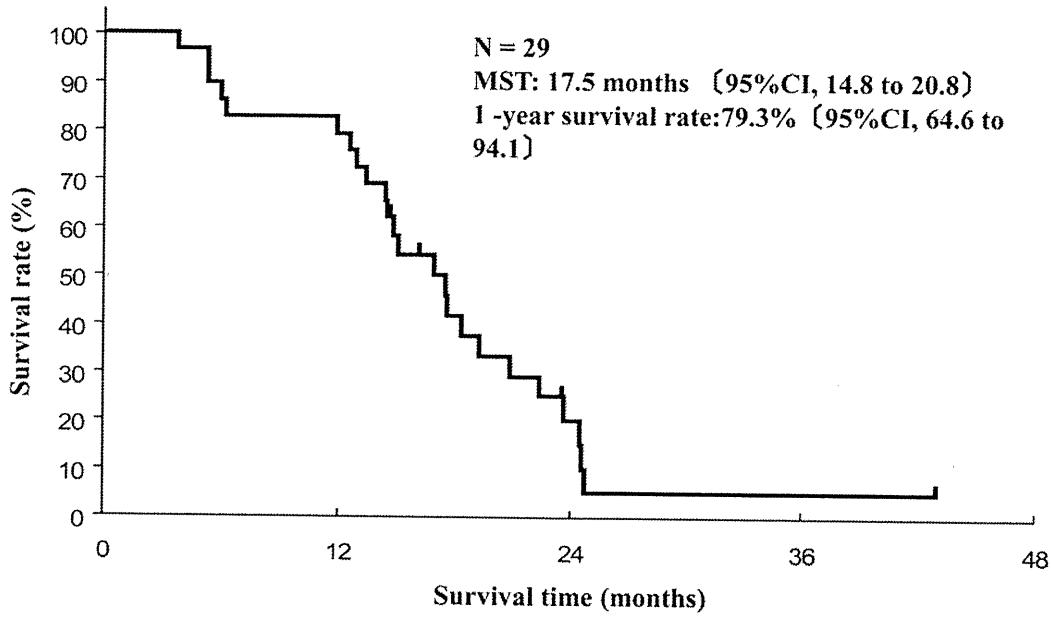

Figure 2. *The survival curve for patients treated with topotecan combination with cisplatin on day 5 is shown. MST, Median survival time; 95% CI, 95% confidence interval.*

Table III. *Adverse events of stage 1 in this phase II study.*

	Toxicity*	CDDP on day 1 (n=15)				CDDP on day 5 (n=15)			
		Grade		Any grade		Grade		Any grade	
		3/4 (n)	(%)	(n)	(%)	3/4 (n)	(%)	(n)	(%)
Hematological	Leukopenia	6/0	40	11	73.3	1/0	7	12	80.0
	Neutropenia	3/7	67	12	80.0	4/2	40	14	93.3
	Thrombocytopenia	6/4	67	14	93.3	3/3	60	15	100.0
	Anemia	7/2	60	14	93.3	5/2	47	15	100.0
Hepatic	AST	0/0	0	2	13.3	2/0	13	10	66.7
	ALT	1/0	7	3	20.0	0/0	0	9	60.0
	T-bilirubin	0/0	0	3	20.0	0/0	0	5	33.3
Gastrointestinal	Anorexia	1/0	7	9	60.0	0/0	0	13	86.7
	Nausea	0/0	0	12	80.0	0/0	0	14	93.3
	Vomiting	0/0	0	8	53.3	0/0	0	9	60.0
	Constipation	0/0	0	8	53.3	0/0	0	10	66.7
	Diarrhoea	0/0	0	1	6.7	0/0	0	5	33.3
	Hiccup	0/0	0	6	40.0	0/0	0	2	13.3
Systemic	Fatigue	0/0	0	10	66.7	1/0	7	10	66.7
	Body weight loss	0/0	0	5	33.3	1/0	7	7	46.7
Fever and other	Fever	0/0	0	2	13.3	0/0	0	1	6.7
	Alopecia	0/0	0	12	80.0	0/0	0	11	73.3

*Severity of each event was evaluated according to CTCAE version 3.0.

schedule group was 83%, including 1 CR. The median survival time was 17.5 months and the 1-year survival rate was 79%. Among observed adverse events, grade 3/4 events were hematological. Major non-hematological adverse events were digestive organ toxicity, (anorexia, nausea, vomiting and constipation), and alopecia, with grade 1/2. These events were also observed in Tp mono therapy (20), thus no CDDP contribution of to stimulation of toxicity was considered. However, AST/ALT increases did occur, in 50% of CDDP day 5 schedule cases. These hepatic events were transient

Table IV. *Adverse events of stage 2 in this phase II study.*

		Grade		Any grade	
	Toxicity*	3/4 (n)	(%)	(n)	(%)
Hematological	Leukopenia	4/0	13.8	26	89.7
	Neutropenia	8/6	48.2	28	96.6
	Thrombocytopenia	5/7	41.4	27	93.1
	Anemia	14/3	58.6	29	100.0
Hepatic	AST	2/0	6.9	15	48.2
	ALT	0/0	0	17	58.6
	T-bilirubin	0/0	0	7	24.1
Gastrointestinal	Anorexia	0/0	0	26	89.7
	Nausea	0/0	0	25	86.2
	Vomiting	0/0	0	15	51.7
	Constipation	0/0	0	20	69.0
	Diarrhoea	0/0	0	10	34.5
	Hiccup	0/0	0	11	37.9
Systemic	Fatigue	1/0	3.4	21	72.4
	Body weight loss	1/0	3.4	12	41.4
Fever and other	Fever	0/0	0	5	17.2
	Alopecia	0/0	0	21	72.4

Severity of each event was evaluated according to CTCAE version 3.0.

and did not influence on study continuation. Similar incidence of such as increase was reported for irinotecan (4). Since the total treatment of completed cycles for both stages 1 and 2 was expected to be 4, high tolerability was estimated. The response rate to Tp combination with CDDP in this study was very much improved compared with the 39% response to Tp mono therapy (21). On the other hand, no difference in the toxic profile was observed, suggesting that the contribution of CDDP was solely for efficacy. The toxicity difference between two schedules is considered as the influence on renal function disorder caused by CDDP as observed in the regimen of CDDP day 1 schedule (7).

The response rate of this combination was equally matched to that of irinotecan in combination with CDDP, but superior in median survival time and 1-year survival rate. Furthermore, this regimen had a better profile for incidence and grade of diarrhea than that of irinotecan.

The median survival times and 1-year survival rate in this study were similar to those obtained in Japanese patients with irinotecan regimen. Noda *et al.* showed survival benefit for CDDP plus irinotecan in comparison with PE (4). However, two similar trials (22, 23) did not show any benefit of irinotecan in combination with CDDP over PE. The sample size or ethnic effect has been considered as the reason for this difference (22). Taking these studies together, irinotecan is an active drug for SCLC in some populations and settings. Superior results of Topo I inhibitor and CDDP regimens in Asian individual, Japanese, and Korean, have been reported (24-26).

The clinical efficacy of Topo I inhibitor plus CDDP is summarized in Table V. The response rates, median survival times and survival rates of patients treated with Tp plus CDDP and irinotecan plus CDDP regimens are better than the combination of etoposide plus CDDP. Since it is known that the efficacies of etoposide and CDDP combination in Japan and North America/Europe are similar, there may be difference in response to the CDDP combination with Topo I inhibitor or with Topo II inhibitor in Japanese patients. Recently, an ethnic effect for response to chemotherapy, such as the gefitinib study, has been revealed (27). These results suggest the possibility of an ethnic effect in non-small cell lung cancer therapy, and study of such as effect may lead to a novel therapy against ED-SCLC. Tp, when compared with irinotecan, has a lower rate of associated diarrhea. Therefore, Tp combined with CDDP therapy resulted in a higher response rate and survival time from this study. It is possible that this combination therapy regimen of Tp for previously untreated ED-SCLC patients may be as useful as irinotecan combination with CDDP therapy.

Conclusion

The combination of Topo I inhibitor, Tp on 5 consecutive days and CDDP on day 5 with G-CSF support is a safe and active regimen for therapy-naive Japanese patients with ED-SCLC. This regimen appeared to be well-tolerated in this patient population. Future clinical trials should elucidate the role of Tp in first-line treatment of ED-SCLC.

Acknowledgements

This study was supported by an unrestricted grant from Nippon Kayaku Co., Ltd., Tokyo, Japan.

References

1 Adjei AA, Marks RS and Bonner JA: Current guidelines for the management of small cell lung cancer. Mayo Clin Proc *74*: 809-816, 1999.

2 Pujol JL, Carestia L and Daures JP: Is there a case for cisplatin in the treatment of small-cell lung cancer? A meta-analysis of randomized trials of a cisplatin-containing regimen *versus* a regimen without this alkylating agent. Br J Cancer *83(1)*: 8-15, 2000.

3 Sundstrom S, Bremnes RM, Kaasa S, Aasebo Ulf, Hatlevoll R, Dahle R, Boye B, Wang M, Vigander T, Vilsvik J, Skovlund E, Hannisdal E and Aamdal S: Cisplatin and etoposide regimen Is superior to cyclophosphamide, epirubicin, and vincristine regimen in small-cell lung cancer: Results from a randomized phase III trial with 5 years' follow-up. J Clin Oncol *20(24)*: 4665-4672, 2002.

4 Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, Fukuoka M, Mori K, Watanabe K, Tamura T, Yamamoto S and Saijo N: Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. N Engl J Med *346(2)*: 85-91, 2002.