The grades were based on a previously reported protocol (Kawase et al. 2008). The immunostaining score was evaluated based on the staining intensity and the percentage of cancer cells that showed positive staining. The following scoring system was used: 0 (negative staining, defined as no immunoreactivity); 1+ (weak staining intensity); and 2+ (strong staining intensity). We also evaluated the extent of staining in a lesion as a percentage (0–100 %). The staining scores were calculated by multiplying the percentage values by the staining intensity, with the scores ranging from 0 to 200. We confirmed that the positive control tissues were stained by each antibody, and we also performed negative control studies without the primary antigen for all the antibodies.

Immunohistochemical scores of cancer cells within PDPN-CAFs (+) areas and PDPN-CAFs (−) areas

We selected 20 adenocarcinomas with a PDPN-CAF expression grade of 1, which had sparsely PDPN-CAF (+) areas. One or two PDPN-CAFs (+) and PDPN-CAFs (−) areas (objective lens 20×; 0.24 mm^2) were selected, and the immunoreactivity of the cancer cells in each area was examined. The average staining score was determined, and the results were recorded as the score for that case.

Cell culture and reagents

The human lung adenocarcinoma cell line PC-9 was purchased from the European Collection of Cell Culture. The PC-9 cell lines were maintained in RPMI 1640 (Sigma-Aldrich, St. Louis, MO) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Nichirei Bioscience, Tokyo, Japan), 1 % glutamine (Sigma-Aldrich), and antibiotics (1 % penicillin and streptomycin [Sigma-Aldrich]). The cultures were incubated at 37 °C in an atmosphere containing 5 % CO_2.

Lentiviral vectors

For the Ezrin shRNA experiments, oligonucleotides were chemically synthesized. Oligonucleotides encoding both strands of the targeting sequence (Supplementary Table 2) were annealed and inserted into the *BglII*/*XbaI* sites of pENTR4-H1 (RIKEN BioResource Center, Japan). A cassette containing the H1 promoter plus the shRNA was transferred to a self-inactivating (SIN) LV construct using Gateway® LR ClonaseII™ Enzyme Mix (Invitrogen), generating CS-H1-shRNA-CG. Then, 293T cells were transfected with 3 plasmids: PCAG-HIV, pCMV-VSV-G-RSV-Rev (RIKEN BioResource Center), and CS-H1-shRNA-CG. The transfection was achieved using Lipofect AMINE 2000 reagent (Invitrogen) according to the manufacturer's instructions. Virus-containing medium was filtered through a 0.45-mm filter, and 8 μg/mL (final concentration) of polybrene (Sigma) was added for target cell transduction. The transduction efficiency was evaluated using a flow cytometry analysis to detect the positivity of enhanced green fluorescent protein (EGFP), the expression of which was under the control of the CMV gene promoter.

Western blot

The Western blot analysis was performed as previously reported (Hoshino et al. 2011). The blots were incubated overnight at 4 °C with antihuman mouse monoclonal Ezrin antibody (Cell signaling Technology, Inc.). After washing in TBS-T, the membranes were incubated with HRP-rabbit anti-mouse IgG (Zymed). ECL Western Blotting Detection Reagents (GE Healthcare) were used to develop the high-performance chemiluminescence film (GE Healthcare).

Wound healing assay

A single scratch was made in the monolayer using a micropipette tip. Subsequently, the cells were washed and then incubated with growth medium. After 7 h of observation, we measured the area occupied by the cells healing the wound and calculated the invasion rate.

Matrigel invasion assay

A Matrigel invasion assay was performed using 24-well culture chambers and a growth factor-reduced, Matrigel-coated filter with a pore size of 8 μm (Becton–Dickinson Labware). The lower chamber contained 0.6 mL of RPMI-1640 + 10 % FBS. In the upper compartment, 2×10^4 of shLuc or shEzin-induced PC-9 cells were placed in triplicate wells and were incubated for 24 h. After incubation, the cells that had passed through the filter were stained with hematoxylin and were counted under a microscope in 9 predetermined fields (950 × 650 μm/field).

Statistical analysis

For the univariate analysis, the Pearson chi-square test was used to determine the statistical significance of the differences between two groups. For the staining scores, the Mann–Whitney *U* test was used because it did not have a normal distribution. For pathological factors such as vascular invasion, lymphatic permeation, and pleural invasion, the differences in variables were evaluated using the log-rank test. All of the reported *p* values were two-sided, and the significance level was set at $p < 0.05$. All the analyses were performed using SPSS Statistics version 21.0 for Windows (SPSS, Chicago, IL, USA).

Table 1 Relationship between grade of podoplanin expression in CAFs and clinicopathological characteristics

Variables	The number of cases			*p* value		
	PDPN-CAFs grade 0 ($n = 65$)	PDPN-CAFs grade 1 ($n = 34$)	PDPN-CAFs grade 2 ($n = 20$)	Grade 0 vs 1	Grade 1 vs 2	Grade 0 vs 2
Gender						
Male	36	21	13	0.67	0.81	0.61
Female	29	13	7			
Age						
≥70	32	15	10	0.68	0.78	0.95
<70	33	19	10			
Smoking status (B.I.)						
≥400	29	19	9	0.30	0.57	0.97
<400	36	15	11			
Node metastasis						
n (+)	2	6	6	0.02	0.32	<0.01
n (−)	63	28	14			

PDPN-CAFs podoplanin-expressing cancer-associated fibroblast, *B.I.* Brinkmann index

Results

Relationship between grade of podoplanin expression in CAFs and clinicopathological characteristics in adenocarcinoma patients with pathological lesions of 2–3 cm in diameter

Table 1 shows the relationship between the grade of PDPN expression in CAFs and the clinicopathological characteristics of the 119 adenocarcinoma patients with pathological lesions of 2–3 cm in diameter. Sixty-five patients (54.6 %) were PDPN-CAF-negative (grade 0) (Fig. 1a), 34 (28.6 %) were PDPN-CAF-positive with a grade of 1, and 20 (16.8 %) were PDPN-CAF-positive with a grade of 2 (Fig. 1b).

In a univariate analysis, the PDPN-CAF expression grade (podoplanin-positive CAF area/stromal area × 100) was significantly associated with the rate of node metastasis (grade 0 vs. grade 1: $p = 0.02$, grade 0 vs. grade 2: $p < 0.01$) (Table 1). We also evaluated the local invasiveness of cancer cells, including vascular invasion, lymphatic permeation, and pleural invasion. Vascular invasion (grade 0 vs. grade 2: $p < 0.01$) significantly increased with an increased grade of PDPN expression in CAFs. For pleural invasion, there was a borderline significance (grade 0 vs. grade 2: $p = 0.05$) (Fig. 1c). These results were partly consistent with our previous reports of stage I adenocarcinoma cases (Ito et al. 2012a).

Phenotypical differences of cancer cells in PDPN-CAFs (+) adenocarcinoma and PDPN-CAFs (−) adenocarcinoma

Since a correlation between PDPN-CAFs (+) and the increased invasive and metastasis abilities of cancer cells was confirmed, we examined the expression of invasion-related molecules, such as adhesion molecule and the epithelial-mesenchymal transition (EMT) markers, of cancer cells with grade 2 PDPN-CAFs (+) surrounding areas and grade 0 PDPN-CAFs (−) surrounding areas ($n = 20$, each). The clinicopathological characteristics of the extracted cases are shown in Supplementary Table 3. The results of the staining scores for the cancer cells in the grade 0 and grade 2 PDPN-CAF cases and the significance of these scores in univariate analyses are shown in Table 2. The Ezrin and E-cadherin scores of the cancer cells were significantly higher in the PDPN-CAF grade 2 cases (Fig. 2b, d) than in the grade 0 cases (Fig. 2a, b) (Ezrin: 32.5 vs. 73, E-cadherin: 57 vs. 93). The median staining scores of the other molecules did not show any significant differences.

Phenotypical differences in cancer cells between PDPN-CAFs (+) areas and PDPN-CAFs (−) areas within the same tumor

To validate the anatomical correlation between Ezrin and E-cadherin-overexpressed cancer cells and PDPN-CAF, we examined the Ezrin and E-cadherin expressions in cancer cells within PDPN-CAFs (+) areas (Fig. 3c) and PDPN-CAFs (−) areas (Fig. 3d) within the same tumor using 20 PDPN-CAF grade 1 cases (Fig. 3a, b). Of the 2 antibodies, only the Ezrin staining score for cancer cells within PDPN-CAFs (+) areas (Fig. 3e) was significantly higher than that for cancer cells within PDPN-CAFs (−) areas (Fig. 3f) (score of 70 vs. 42.5, $p < 0.01$) (Table 3). No significant differences in the staining scores for E-cadherin were observed.

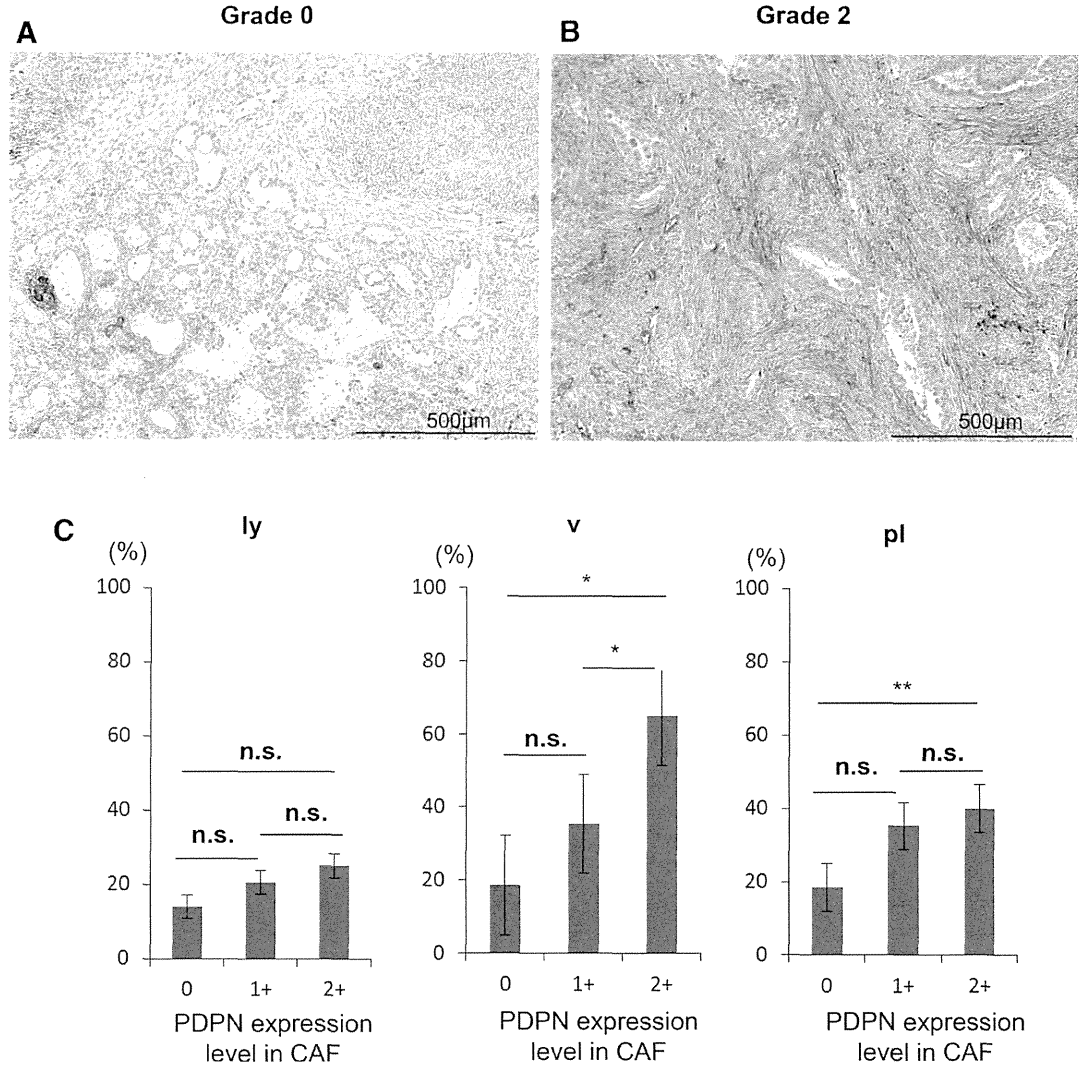

Fig. 1 **a**, **b** Grading for PDPN-CAFs. **a** Grade 0: no PDPN-CAFs are present in the invasive area of the adenocarcinoma. **b** Grade 2: PDPN-CAFs are found in 50–100 % of the invasive area of the adenocarcinoma. **c** Frequency of lymphatic permeation, vascular invasion, and pleural invasion according to the PDPN-CAF grade in 119 adenocarcinoma patients. Vascular invasion significantly increased with an increased grade of PDPN expression in CAFs (*$p < 0.05$). For pleural invasion, there was a borderline significance (**grade 0 vs. grade 2: $p = 0.05$)

Ezrin-knockdown lung adenocarcinoma cells exhibited lower migration and invasive activities

To examine whether Ezrin expression in lung adenocarcinoma cells is involved in cell migration and invasiveness, we generated Ezrin-knockdown PC-9 cells. Short hairpin RNA for Ezrin or luciferase was transduced into PC-9 cells. The expression levels of Ezrin in the transduced cells were confirmed using a Western blot analysis (Supplementary Figure 1) and RT-PCR (data not shown). In the wound healing assay, the migration rate of each shEzrin (shEzrin 1 to shEzrin 3)-induced PC-9 cell line was significantly lower than that of a control. Moreover, in a Matrigel invasion assay, the invasive activities of the shEzrin-induced PC-9 cell lines were also significantly lower than those of the control cells (Fig. 4).

Discussion

We previously reported that PDPN-CAFs were associated with a tumor-promoting phenotype in both in vitro and in vivo studies (Hoshino et al. 2011). The current study using adenocarcinomas of relatively uniform size showed that

Table 2 Staining score of cancer cells in PDPN-CAFs (−) and PDPN-CAFs (+) cases

Category	Antibody	Staining score of cancer cells[a]		*p* value
		PDPN-CAFs grade 0	PDPN-CAFs grade 2	
Cell adhesion and invasion	Integrin β1	10 (0–82)	34 (0–132)	0.16
	Laminin 5	26 (4–92)	41 (4–100)	0.41
	CD44	20 (0–88)	40 (0–180)	0.14
	Ezrin	32.5 (0–70)	73 (20–100)	<0.01
Growth factor receptor	EGFR	3 (0–56)	5 (0–78)	0.43
	cMET	75 (14–134)	52 (10–128)	0.19
EMT	E-Cadherin	57 (20–92)	93 (14–170)	<0.01
	Fibronectin	0 (0–24)	0 (0–76)	0.28
	Clusterin	4 (0–52)	12 (0–136)	0.46
	Caveolin	0 (0)	0 (0–56)	0.29

PDPN-CAFs podoplanin-expressing cancer-associated fibroblast, *EMT* epithelial-mesenchymal transition

[a] Median (range)

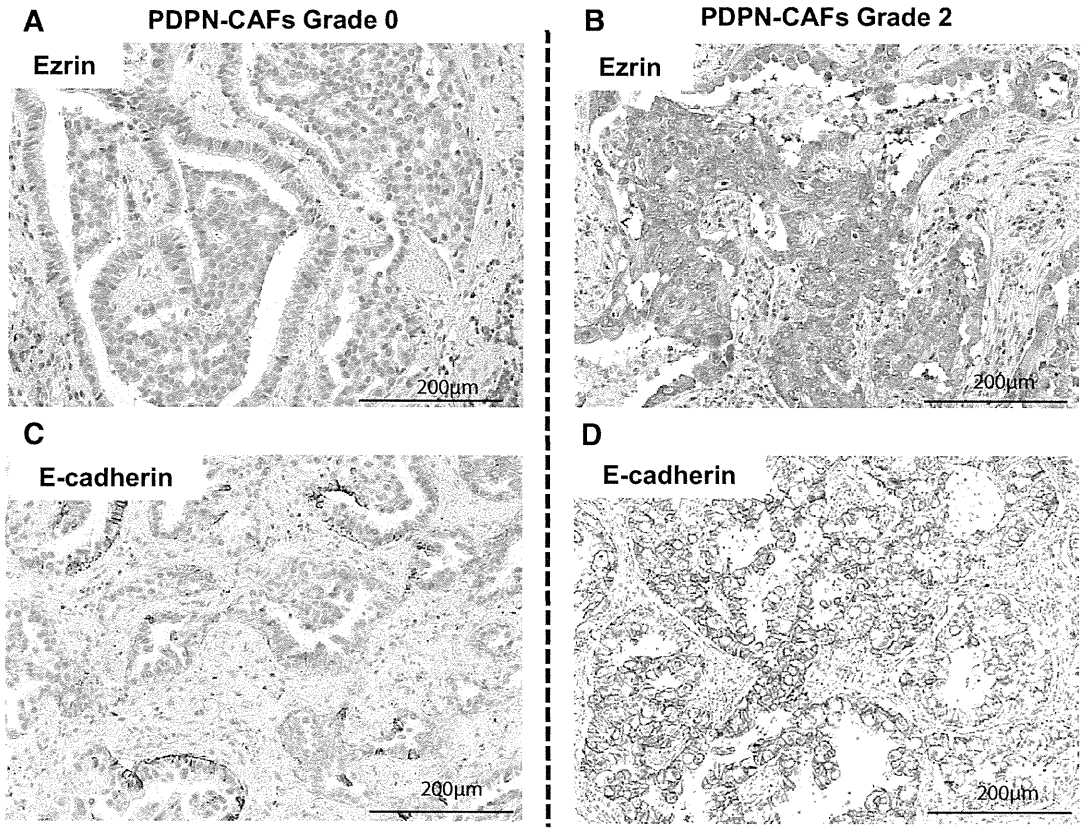

Fig. 2 Phenotypical differences in cancer cells in PDPN-CAFs (+) adenocarcinoma and PDPN-CAFs (−) adenocarcinoma. **a** Ezrin expression in cancer cells in PDPN-CAFs (−) adenocarcinoma. **b** Ezrin expression in cancer cells in PDPN-CAFs (+, grade 2) adenocarcinoma. **c** E-cadherin expression in cancer cells in PDPN-CAFs (−) adenocarcinoma. **d** E-cadherin expression in cancer cells in PDPN-CAFs (+, grade 2) adenocarcinoma

vascular invasion, pleural invasion, and node metastasis were associated with an increased grade of PDPN expression in CAFs, which was partly consistent with our previous clinicopathological reports (Kawase et al. 2008; Ito et al. 2012a). These results suggested that adenocarcinoma cells coexisting with PDPN-CAFs have a high malignant potential, such as invasiveness. In light of these results, we examined invasiveness-related immunohistochemical

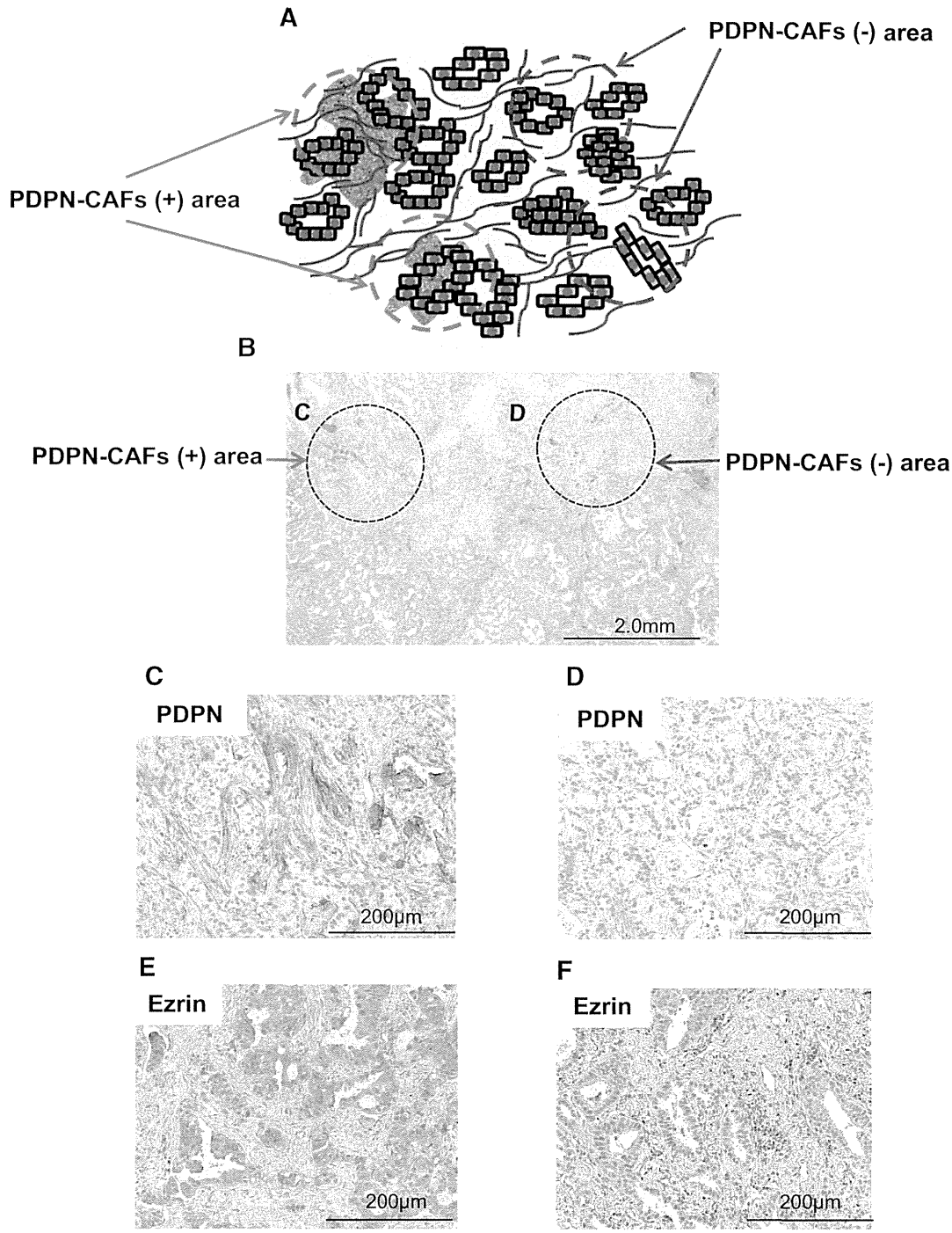

Fig. 3 Phenotypical differences between cancer cells in PDPN-CAFs (+) area and PDPN-CAFs (−) area within the same tumor. **a** Schematic representation of PDPN-CAFs (+) area. **b** PDPN-CAFs grade 1 case which has PDPN-CAFs (+) area and PDPN-CAFs (−) area within the same tumor. **c** Area in which CAFs expressed PDPN. **d** Area in which CAFs did not express PDPN. **e** Ezrin expression in cancer cells within area (**c**). **f** Ezrin expression in cancer cells within area (**d**)

characteristics of cancer cells coexisting with PDPN-CAFs and found an anatomical correlation between PDPN-CAFs and Ezrin-expressing cancer cells. This is the first report to investigate the phenotypes of cancer cells in a microenvironment composed of a specific subpopulation of CAFs, i.e., PDPN-CAFs, and to suggest the implications of microenvironmental heterogeneity within lung adenocarcinoma.

Table 3 Staining scores of cancer cells in PDPN-CAFs negative area and positive area

Antibody	Staining score of cancer cells around CAFs[a]		*p* value
	PDPN-CAFs negative area	PDPN-CAFs positive area	
Ezrin	42.5 (0–100)	70 (30–100)	<0.01
E-Cadherin	72.5 (30–180)	80 (20–185)	0.76

PDPN-CAFs podoplanin-expressing cancer-associated fibroblast

[a] Median (range)

Ezrin is a member of the ERM (Ezrin–Radixin–Moesin) protein family, which provides a physical link between F-actin and cell membrane-associated proteins (Bretscher et al. 2002; Louvet-Vallee 2000; McClatchey 2003). ERM proteins are associated with several adhesion molecules, such as CD44, and their functions are essential for fundamental cellular processes, including cell adhesion and motility (Pujuguet et al. 2003; Tsukita et al. 1994; Xu and Yu 2003). Reportedly, Ezrin is strongly expressed in many types of tumors (Cui et al. 2010; Di Cristofano et al. 2010; Ma et al. 2013; Tynninen et al. 2004; Zhang et al. 2012). In

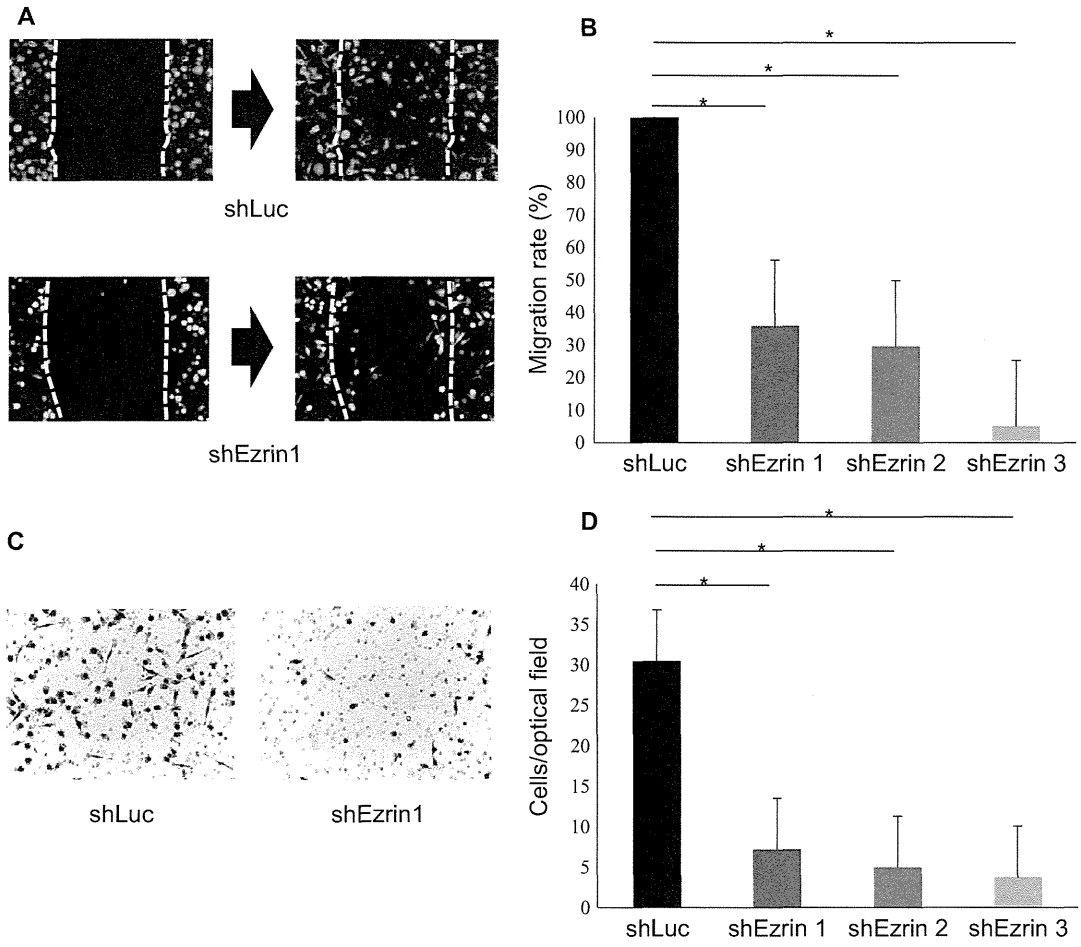

Fig. 4 Wound healing assay and Matrigel invasion assay using Ezrin-knockdown PC-9 cells. **a** Wound healing assay. The green cells are shLuciferase or shEzrin 1-induced PC-9 cells at 7 h after well scratching. The white dotted lines represent the scratch lines. **b** Migration rate of the cells into the central area of the wound as an indicator of migration activity using shEzrin 1 to 3-induced PC-9 cells or shLuciferase-induced PC-9 cells. **c** Matrigel invasion assay. shLuciferase-induced or shEzrin 1-induced PC-9 cells that had passed through a filter are shown. **d** Number of cells that had invaded through the filter into the lower chamber as an indicator of invasive activity using shEzrin 1 to 3-induced PC-9 cells or shLuciferase-induced PC-9 cells. $*p < 0.05$

lung cancer, Ezrin expression in cancer correlated significantly with lymphatic metastasis and advanced TNM stage (Li et al. 2012). Similarly, in our study, Ezrin expression in cancer cells ("positive" was judged as over 50 % of cancer cells) showed higher tendency of pleural invasion (positive vs. negative: $p = 0.05$) (Supplementary Table 4).

In lung adenocarcinoma, the high level of Ezrin expression is reportedly associated with wide therapeutic applications in the treatment of human lung cancer in studies using the human lung carcinoma cell line 95D, with connections to the promotion of morphological change, migration, growth, and invasiveness (Chen et al. 2013). These findings are consistent with our present in vitro results. Therefore, Ezrin is thought to be a key regulatory molecule in invasiveness. These findings may partly explain why PDPN-CAF (+) adenocarcinoma cases had higher degrees of vascular invasion, node metastasis, and pleural invasion.

Activated Ezrin in cancer cells can link various plasma membrane proteins to the actin cytoskeleton (Fehon et al. 2010). Activated Ezrin also binds and sequesters RhoGDI (Rho GDP-dissociation inhibitor) (Hirao et al. 1996; Maeda et al. 1999; Takahashi et al. 1997), thereby initiating the activation of RhoA and maintaining Ezrin activation. On the other hand, we previously reported that PDPN-expressing fibroblasts exhibited higher levels of RhoA activity (Ito et al. 2012b). Taking these into consideration, RhoA activity might increase in both PDPN-CAFs and Ezrin-expressing cancer cells within the same area; therefore, Rho-inhibitor might appear to have great potential as a new therapeutic target for cancer microenvironment composed of PDPN-CAFs.

Meanwhile, the molecular mechanisms responsible for inducing the expression of PDPN in CAFs and the expression of Ezrin in cancer cells have been unclear. As a PDPN expression-promoting factor, TGF-β1 has been reported in an oral squamous cell carcinoma cell line (Ohta et al. 2013) and a human fibrosarcoma cell line, HT1080 (Suzuki et al. 2008). TGF-β1 has also been reported to be an Ezrin expression-promoting factor in human trophoblast derived from placentae and the human choriocarcinoma cell line JEG-3 (Karmakar and Das 2004). A tumor microenvironment composed of PDPN-CAFs and Ezrin-expressing cancer cells might exist under TGF-β1-rich conditions. In lung cancer, Hasegawa et al. (2001) reported a significant correlation between TGFβ1 expression level and poor prognosis in patients with adenocarcinoma, which might also support hypothesis mentioned above. Alternatively, PDPN-CAFs might induce Ezrin expression in cancer cells or vice versa; this issue will require further examination in the future.

In conclusion, our study demonstrated that Ezrin expression is a characteristic phenotype of cancer cells in microenvironments containing PDPN-CAFs in lung adenocarcinoma. The current results also indicate the presence of microenvironmental heterogeneity within solid tumors and suggest the biological significance of the unique microenvironment created by Ezrin-overexpressing cancer cells and PDPN-overexpressing CAFs.

At present, not only cancer cells, but also CAFs and their interactions are attracting attention as potential therapeutic targets (Hofheinz et al. 2003; Kraman et al. 2010; Scott et al. 2003). Our discoveries in the present study might serve as a foundation for the development of promising cancer therapies targeting for both cancer cells and CAFs.

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Conflict of interest The authors have no conflict of interest to disclose.

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Prognosis of Lung Cancer Patients with a Past History of Colorectal Cancer

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Objectives: The prognosis in lung cancer patients with a prior history of extrapulmonary cancer is controversial. In the current multicenter joint research in Japan, we focused on the relationship between a history of colorectal cancer and its prognostic impact in patients with subsequent lung cancer.

Methods: Between 2000 and 2013, we designed a retrospective multicenter study at three institutes in Japan to evaluate the prognostic factors in lung cancer patients with a previous surgery for colorectal cancer.

Results: The cohorts consisted of 123/4431 lung cancer patients with/without a previous history of surgery for colorectal cancer. The median follow-up period was 6.1 years after lung cancer surgery. The 5-year overall survival in lung cancer patients with/without colorectal cancer was not significantly different, regardless of the stage of lung cancer (overall: 71.3 versus 74.7%, $P = 0.1426$; Stage I lung cancer: 83.3 versus 84.8%, $P = 0.3779$; Stage II or more lung cancer: 47.7 versus 54.4%, $P = 0.1445$). Based on multivariate Cox regression analysis in 4554 lung cancer patients, a past history of colorectal cancer was not a significant prognostic factor ($P = 0.5335$). Among the 123 lung cancer patients with colorectal cancer, age and absence of adjuvant chemotherapy for colorectal cancer were significant prognostic factors based on multivariate analysis ($P = 0.0001$ and 0.0236). Furthermore, there was no difference in the overall survival of lung cancer patients according to the stage of colorectal cancer (Stage I: 74.7%; Stage II/III: 66.5%, $P = 0.7239$).

Conclusions: A history of antecedent colorectal cancer did not contribute to the prognosis in patients with subsequent lung cancers.

Key words: lung cancer – colorectal cancer – multiple primary cancers – prognosis

INTRODUCTION

Lung cancer is the most frequent cause of major cancer and the leading cause of death worldwide (1,2). Due to recent developments in imaging technology and the widespread use of thin-section computed tomography (CT) for the screening of lung cancer, it is expected that the incidence of lung cancer will increase as a direct result of screening examinations (3). Furthermore, this will lead to an increase in the identification of early-stage lung cancers and a consequent later decrease in mortality.

The risk of developing a new malignancy in patients with an unrelated previous cancer has been reported to be 5–15

times higher than that in the general population (4). As a result of the increasing incidence of any cancers due to improved treatment modalities and long-term survival worldwide (5), we often encounter newly detected lung cancer patients with a prior history of extrapulmonary cancers, especially gastrointestinal lesions (6–10). Among these, colorectal cancer accounts for a high percentage of both incidence and death worldwide and is associated with lung cancer as a multiple primary malignancy (11–13). Many studies have reported several clinical, radiological, pathological and molecular factors that predict the prognosis in lung cancer patients (2,14,15). However, despite remarkable advances in our understanding of lung cancer over the past decade, it is not yet clear whether a history of previous treatment for any other cancers, especially colorectal cancers, affects the prognosis of surgery for lung cancer. In this study, we highlighted colorectal cancer, because it is a common malignancy and fatality rates are high (13). The incidence of multiple primary cancers in patients with colorectal cancer is ~15–20%, and the incidence of lung cancer is the second next to stomach cancer of these patients in the past literature (12). On the other hand, a few studies have addressed the clinical behavior and survival of small cohorts of patients with non-small cell lung cancer who had previously been treated for colorectal cancer. Thus, we evaluated the survival and prognostic factors of lung cancer patients with the past history of colorectal cancer.

At any single institution, the number of patients with a previous history of colorectal cancer is relatively small. Therefore, the purpose of this study was to investigate the clinicopathological impact of preceding surgery for colorectal cancer in patients with lung cancer by multi-institutional joint research in Japan.

PATIENTS AND METHODS

Approval

This was a multi-institutional joint research study (Juntendo University School of Medicine, IRB No. 13–133; National Cancer Center Hospital East, IRB No. 2013-106; Hiroshima University Hospital, IRB No. eki-862). The study protocol was approved by the institutional review boards of the three participating institutes. Due to a retrospective study, the need to obtain written informed consent from each patient was waived.

Patients

Based on their cancer history, patients were categorized as either lung cancer patients with previous surgery for colorectal cancer, or those without a history of colorectal cancer. A history of colorectal cancer was confirmed when they were diagnosed or treated for lung cancer at our institutes, otherwise documentation was obtained from other hospitals or clinics. Between January 2000 and June 2013, there were 123 lung cancer patients with a history of preceding surgery for colorectal cancer at our three institutes. The sufficient information regarding their history of colorectal cancer was included in the primary analysis as lung cancer patients with a history of preceding surgery for colorectal cancer. In contrast, we enrolled 4431 lung cancer patients without a history of colorectal cancer as a control group. All patients underwent surgical operation for lung cancer at each institute.

Demographic Data

In all cases, the medical record of each patient was reviewed with regard to age, gender, serum carcinoembryonic antigen level (ng/ml, CEA) and several clinicopathological characteristics to evaluate the prognostic factors and elucidate whether the treatment history for colorectal cancer influences the prognosis of subsequent lung cancer surgery. The clinical and pathological stages of each disease were determined based on the International Union Against Cancer, 7th edition (16).

Operation Policy

Regarding the operative modes for lung cancer, each institute had a consensus that major lung dissection with systemic lymph node dissection is the standard procedure for resectable non-small cell lung cancer despite a previous history of colorectal cancer. Segmentectomy is indicated in part for lung cancers 2 cm or less in size with ground-glass opacity dominant lesion. Nonanatomic wedge resection is also performed for a few elderly patients or for patients with high cardiopulmonary risk.

Statistics

In the statistical analysis, the Chi-square test or unpaired *t*-test were used to compare two factors. Cumulative survival rates for each group, i.e. lung cancer patients with or without a history of colorectal cancer, were calculated by the Kaplan–Meier method, where the date of surgical resection for lung cancer was used as the starting point and the date of death due to any cause or the date of the last follow-up was used as the end point. The interoperative interval was calculated from the date of surgical resection for colorectal cancer to that for lung cancer. Univariate and multivariate analyses were used to identify clinicopathological factors that significantly predicted the prognosis in patients with or without preceding surgery for colorectal cancer. A univariate analysis was performed by the log-rank test. A multivariate analysis was performed by the Cox proportional hazard model using SPSS Statistics 21 (SPSS Inc.). Forward and backward stepwise procedures were used to determine the combination of factors that were essential for predicting the prognosis. Continuous data are shown as the mean and standard deviation for normality. The results of the statistical analysis were considered to be significant when the probability value was <0.05.

RESULTS

The clinicopathological characteristics of the entire population are shown in Table 1. The average age was 70 years (range, 46–89) in patients with colorectal cancer, and 66 years (range, 20–93) in those without colorectal cancer ($P < 0.0001$). Among the 123 lung cancer patients with colorectal cancer, 91 (74.0%) were men and 32 (26.0%) were women, whereas among the 4431 patients without colorectal cancer, 2713 (61.2%) were men and 1718 (38.8%) were women, and this was a significant difference between the groups ($P = 0.0041$). With regard to the clinical stage of lung cancer, the patients with the past history of colorectal cancers showed significantly early-stage disease of lung cancer ($P = 0.0039$). A previous history of any organ cancer, including colorectal cancer, was observed in 473 (10.4%) of the total patients. The median follow-up period among the overall patients was 6.1 years after lung cancer surgery (range, 0–11.8 years). In lung cancer patients with a past history of colorectal cancer, the median time interval from the date of operation for colorectal cancer to that for lung cancer was 2.4 years (range, 0–25.1 years). The frequencies of adjuvant chemotherapy for lung cancer and the absence of malignancies other than lung and colorectal cancer were significantly higher in lung cancer patients without a history of colorectal cancer ($P = 0.0014$ and 0.0022, respectively).

Based on univariate and multivariate analyses in 4554 patients with surgically resected lung cancers, gender, age at lung cancer surgery, the presence of any cancers other than those of the lung and colorectum, the pathological stage of lung cancer and the administration of adjuvant chemotherapy for lung cancer were significant prognostic factors in this population, whereas a history of surgery for colorectal cancer was not a predictor in multivariate analysis ($P = 0.7793$) (Table 2).

The overall survival (OS) curves of the populations with and without colorectal cancer are presented in Fig. 1. As shown in Table 2, significant differences were not observed between these two groups; however, the 5-year OS in lung cancer patients with colorectal cancer (71.3%) was slightly inferior to that in patients without colorectal cancer (74.7%) ($P = 0.1426$). Moreover, we evaluated the OS of lung cancer patients with and without colorectal cancer based on the lung cancer staging. According to the results, the OS was not significantly different regardless of the presence of colorectal cancer in patients with both pathological Stage I and Stage II or more lung cancer [Stage I lung cancer (Fig. 2, $n = 3092$): with colorectal cancer = 83.3%, without colorectal cancer = 84.8%, $P = 0.3779$; Stage II or more lung cancer (Fig. 3, $n = 1462$): with colorectal cancer = 47.7%, without colorectal cancer = 54.4%, $P = 0.1445$].

The clinicopathological characteristics of lung cancer patients with a previous surgery for colorectal cancer are shown in Table 3. Of the 123 patients, 66 (53.7%) showed colorectal cancer of pathological Stage I. The mean interoperative interval from colorectal cancer to lung cancer was 3.9 ± 4.4 years (range, 0–25.1 years). Adjuvant chemotherapy for colorectal cancer was administrated to 33 (26.8%) patients. With regard to the cause of death, death due to lung

Table 1. Clinicopathological characteristics of all lung cancer patients

Factors	Overall lung cancer patients		*P* value*
	With colorectal cancer (%)	Without colorectal cancer (%)	
Total	123	4431	
Average age (year)	70	66	<0.0001
Gender			
Male	91 (74.0)	2713 (61.2)	0.0041
Female	32 (26.0)	1718 (38.8)	
CEA (ng/ml)	6.8 ± 9.3	8.9 ± 65.4	0.7253
Clinical stage of lung cancer			
IA	74 (60.2)	2463 (55.6)	0.0039
IB	31 (25.2)	1006 (22.7)	
IIA	9 (7.4)	319 (7.2)	
IIB	3 (2.4)	263 (5.9)	
IIIA	3 (2.4)	334 (7.5)	
IIIB	0 (0)	21 (0.5)	
IV	3 (2.4)	25 (0.6)	
Pathological stage of lung cancer			
IA	65 (52.8)	2162 (48.8)	0.5283
IB	23 (18.7)	842 (19.0)	
IIA	7 (5.7)	379 (8.5)	
IIB	12 (9.8)	292 (6.6)	
IIIA	14 (11.4)	603 (13.6)	
IIIB	0	106 (2.4)	
IV	2 (1.6)	47 (1.1)	
Surgical procedures for lung cancer			
Wedge	13 (10.6)	329 (7.4)	0.3306
Segmentectomy	13 (10.6)	315 (7.1)	
Lobectomy	95 (77.2)	3635 (82.0)	
Pneumonectomy	3 (2.4)	152 (3.4)	
Histology of lung cancer			
Adenocarcinoma	90 (73.2)	3173 (71.6)	0.9093
Squamous cell carcinoma	23 (18.7)	899 (20.3)	
Others	10 (8.1)	359 (8.1)	
Adjuvant chemotherapy for lung cancer			
Yes	15 (12.2)	1098 (24.8)	0.0014
No	108 (87.8)	3333 (75.2)	
Cancers other than those of lung and colorectum			
Presence	23 (18.7)	450 (10.2)	0.0022
Absence	100 (81.3)	3981 (89.8)	

CEA, carcinoembryonic antigen.
**P* value in the Chi-square test or unpaired *t*-test.

Table 2. Univariate and multivariate analysis of overall survival in 4554 patients with lung cancer

Covariate	Univariate	Multivariate		
	P value*	HR	95% CI	*P* value*
Gender (female)	<0.0001	0.615	0.527–0.717	<0.0001
Age at lung cancer surgery (year)	<0.0001	1.032	1.025–1.040	<0.0001
CEA (ng/ml)	0.1896	1.000	1.000–1.001	0.2862
Cancers other than those of the lung and colorectum (absence)	<0.0001	0.625	0.515–0.758	<0.0001
Pathological stage of lung cancer (pathological Stage I)	<0.0001	0.234	0.204–0.272	<0.0001
Adjuvant chemotherapy for lung cancer (absence)	<0.0001	0.726	0.617–0.855	0.0001
Surgery for colorectal cancer (absence)	0.1439	0.949	0.730–1.520	0.7793

CI, confidence interval; HR, hazard ratio.
**P* value in the Cox proportional hazard model.

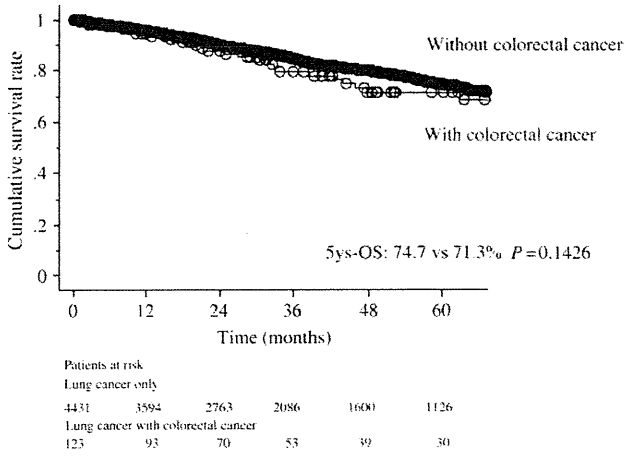

Figure 1. Overall survival curve for all lung cancer patients with and without a previous history of colorectal cancer. A statistically significant difference was not observed between the outcomes of the two groups (log-rank test, $P = 0.1426$). OS, overall survival.

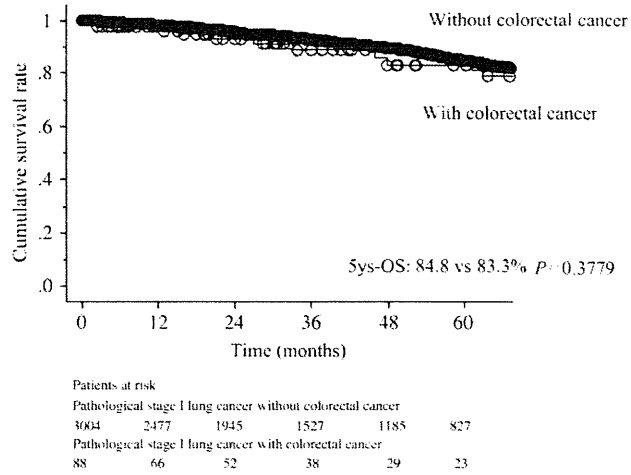

Figure 2. OS curve for pathological Stage I lung cancer patients with and without a previous history of colorectal cancer. A statistically significant difference was not observed between the outcomes of the two groups (log-rank test, $P = 0.3779$).

cancer was observed in 18 patients, whereas only 1 patient died because of the recurrence of colorectal cancer.

Based on multivariate analysis for lung cancer patients with previous surgery for colorectal cancer, age at the operation for lung cancer and the absence of adjuvant chemotherapy for colorectal cancer were significant prognostic factors ($P = 0.0001$ and 0.0236, respectively) (Table 4). We excluded these two prognostic factors from the cohort of patients with colorectal cancer and re-evaluated the OS. Based on the results, the 5-year OS for all lung cancer patients with colorectal cancers, excluding those >76 years old (the 5-year OS = 77.0%, $P = 0.8499$), and excluding those who were given adjuvant chemotherapy for colorectal cancer (the 5-year OS = 76.0%, $P = 0.4434$) compared with those in patients without colorectal cancer (the 5-year OS = 74.7%) were almost equivalent.

With regard to the OS of lung cancer patients with colorectal cancer based on their pathological status, the 5-year OS of lung cancer patients with pathological Stage I colorectal cancer (74.7%) was better than that of lung cancer patients with pathological Stage II or III colorectal cancer (66.5%), although a significant difference was not observed with regard to the stage of colorectal cancer (Fig. 4; $P = 0.7239$). Furthermore, we evaluated the prognostic factors for OS in lung cancer patients with a previous surgery for pathological Stage I colorectal cancer ($n = 66$). Based on multivariate analysis, age at the operation for lung cancer and the recurrence of colorectal cancer were significant prognostic factors in these populations ($P = 0.0298$ and 0.0285, respectively) (Table 5).

DISCUSSION

Colorectal carcinoma is one of the most frequent major cancers that are seen with lung cancer (8,11,12,17), but the

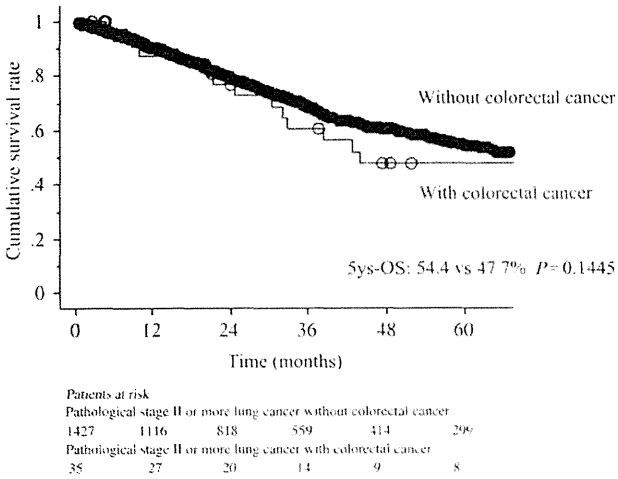

Figure 3. OS curve for pathological Stage II or more lung cancer patients with and without a previous history of colorectal cancer. A statistically significant difference was not observed between the outcomes of the two groups (log-rank test, $P = 0.1445$).

Table 3. Clinicopathological characteristics of lung cancer patients with a previous surgery for colorectal cancer

	Number of patients (%)
Pathological stage of colorectal cancer	
Stage I	66 (53.7)
Stage II or more	57 (46.3)
Interoperative interval from colorectal cancer to lung cancer	
Within 5 years	87 (70.7)
>5 years	36 (29.3)
Treatment methods for colorectal cancer	
Endoscopic resection	30 (24.4)
Surgery	93 (75.6)
Adjuvant chemotherapy for colorectal cancer	
Presence	33 (26.8)
Absence	90 (73.2)
Recurrence of colorectal cancer	
Presence	4 (3.3)
Absence	119 (96.7)
Cause of death	
Lung cancer	18 (62.1)
Colorectal cancer	1 (3.4)
Other diseases	10 (34.5)

clinicopathological features and the impact of past cancer history on the prognosis of lung cancer have been barely validated. Because the number of lung cancer patients complicated with a previous history of colorectal cancers is increasing, there is an urgent need for a study on this topic.

In the current retrospective multicenter study, we focused on the relationship between a history of antecedent colorectal cancer and its prognostic impact in patients with subsequent lung cancer.

With regard to the presence of a past history of any cancers within the previous 5 years in patients with lung cancer, one of the most important points is that this is considered to be an indispensable exclusion criterion in many prospective trials in lung cancer, which could result in a reduction in enrolling the possible candidates. Some reports have indicated that lung cancer patients with preceding cancers in other organs have a poor prognosis (8–10). However, the clinicopathological features of and prognosis in lung cancer patients with a prior history of cancer are not fully resolved yet. Furthermore, recent developments in imaging technology and the widespread use of thin-section CT for screening have made it possible to detect early-stage lung cancers (2,18). Amid a paradigm shift regarding the stage of lung cancer patients, little information is available regarding the prognostic factors in lung cancer patients combined with a previous surgery for some other malignancy, especially for major cancers like cancers of the colon and rectum.

Based on our study, a history of previous surgery for colorectal cancer did not contribute to the prognosis in the overall lung cancer patients. Furthermore, multivariate analysis revealed that age at lung cancer surgery and the absence of adjuvant chemotherapy for colorectal cancer were significant prognostic factors in 123 lung cancer patients with a previous surgery for colorectal cancer. In this study, the frequencies of lung cancer death, colorectal cancer death and cancer non-related death are almost equivalent in lung cancer patients with a past history on colorectal cancer, despite the presence or absence of adjuvant chemotherapy for colorectal cancer. However, the patients administrated adjuvant chemotherapy for colorectal cancer belonged to more advanced stage of colorectal cancer, and the general conditions might be inferior to the patients without adjuvant chemotherapy for colorectal cancer, which influenced to the survival of lung cancer patients. These findings might reflect the poor general status of some colorectal cancer patients performed adjuvant chemotherapy. Interestingly, when these prognostic factors were excluded, the OS rates in lung cancer patients with and without colorectal cancer were almost equivalent by the Kaplan–Meier method. These results obtained from the overall cohort were almost the same as those in lung cancer patients with pathological Stage I colorectal cancer based on the multivariate analysis. Some of the results given above are particularly noteworthy. In particular, a history of colorectal cancer may not contribute to the prognosis in patients with subsequent lung cancers if the lung cancer patients are of a suitable age or if their preoperative status could be well managed by surgery for their previous colorectal cancer.

In a recent study, the pathological stage of colorectal cancer was not a significant predictor in patients with subsequent lung cancer. This is partly due to the retrospective nature of this study, and selection of the candidates for a lung cancer

Table 4. Univariate and multivariate analyses of overall survival in lung cancer patients with the preceding surgery for colorectal cancer

Covariate	Univariate	Multivariate		
	P value*	HR	95% CI	*P* value*
Gender (female)	0.2302	0.744	0.283–1.957	0.5940
Age (year)	0.0376	1.138	1.066–1.215	0.0001
CEA (ng/ml)	0.0067	1.021	0.991–1.053	0.1780
Clinical stage of lung cancer (clinical Stage I)	0.0094	0.411	0.152–1.110	0.0793
Cancers other than those of the lung and colorectum (absence)	0.8868	0.470	0.164–1.343	0.1586
Pathological stage of colorectal cancer (pathological Stage I)	0.7241	0.953	0.288–3.151	0.9375
Surgical procedures for colorectal cancer (surgical resection)	0.7969	0.544	0.193–1.531	0.2487
Adjuvant chemotherapy for colorectal cancer (absence)	0.4086	0.224	0.061–0.818	0.0236
Recurrence of colorectal cancer (absence)	0.1615	0.421	0.084–2.118	0.2942
Intraoperative interval from colorectal cancer to lung cancer (within 5 years)	0.6487	0.827	0.332–2.062	0.6840

**P* value in the Cox proportional hazard model.

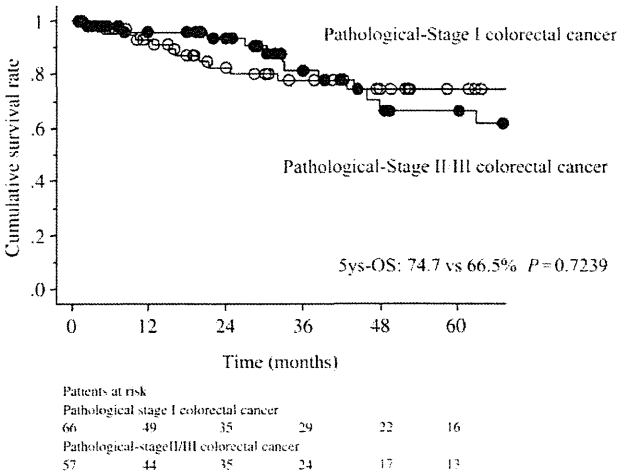

Figure 4. OS curve for lung cancer patients with pathological Stage I or pathological Stage II/III colorectal cancer. A statistically significant difference was not observed between the outcomes of the two groups (log-rank test, $P = 0.7239$).

surgery may be very strict in patients with the history of colorectal cancer. Generally, patients with pathological Stage I or II colorectal cancer have a good prognosis with the 5-year survival rate of ~90% (19). Even pathological Stage IIIA colorectal cancer showed the 5-year survival rate over 70% (19). It is easy to understand that this study population of patients with pathological Stage III colorectal cancers was a relatively minor and may have included many otherwise healthy patients. Furthermore, with the advances in diagnostic modalities such as thin-section CT scan or positron emission tomography, early detection of lung cancer may be possible and also contribute to favorable outcomes by decreasing the risk of uncontrolled colorectal cancer. Therefore, the presence of a past history of colorectal cancer treatment had relatively little effect on lung cancer prognosis if the patients were appropriately selected.

Moreover, with regard to the relationship between a preceding malignancy and subsequent lung cancer, several previous reports described a significant correlation between a history of preceding extrapulmonary malignancy and the early stage of lung cancer (20–22). On the other hand, there seemed to be no close relationship between the pathological stage of colorectal and lung cancer in the current study. If anything, the frequency of pathological Stage I lung cancer in patients with a previous history of pathological Stage II or more colorectal cancers (42/57; 73.7%) was somewhat greater than that in patients with pathological Stage I disease (46/66; 69.7%). These results suggest that more intensive follow-up and examinations are needed for more advanced colorectal lesions compared with early-stage disease.

In this study, the interoperative interval from colorectal cancer to lung cancer did not influence the prognosis of lung cancer patients. We selected 5 years as a cutoff value for the interoperative interval, since this value has often been used as an exclusion criterion in prospective studies worldwide. This lack of a relationship was also supported by multivariate analysis, even when we treated the interoperative interval as a continuous value. These results support our opinion that surgery for lung cancer patients with a previous history of colorectal cancer is feasible in adequately selected cohorts.

A major limitation of this study is its retrospective nature. Patient selection for the surgery of lung cancer may be strict among the presence of a previous history of colorectal cancer in each institute. In addition, although we have standard treatment strategies for lung cancer surgery, the indications for each surgical mode may be somewhat different in each institute in accordance with the patient characteristics. Thus, further prospective studies will be necessary in a larger number of patients. Despite these limitations, however, we

Table 5. Univariate and multivariate analyses of overall survival in lung cancer patients with a previous surgery for pathological Stage I colorectal cancer

Covariate	Univariate	Multivariate		
	P value*	HR	95% CI	*P* value*
Gender (female)	0.6945	1.082	0.243–4.826	0.9176
Age (years)	0.0154	1.105	1.010–1.210	0.0298
CEA (ng/ml)	0.0567	1.018	0.982–1.056	0.3210
Clinical stage of lung cancer (clianical Stage I)	0.0233	0.323	0.075–1.389	0.1286
Malignancies other than colorectal and lung cancer (absence)	0.5198	0.571	0.122–2.667	0.4763
Surgical procedures for colorectal cancer (surgical resection)	0.9631	0.564	0.190–1.672	0.3019
Recurrence of colorectal cancer (absence)	0.0197	0.061	0.005–0.746	0.0285
Intraoperative interval from colorectal cancer to lung cancer (within 5 years)	0.6731	0.988	0.218–3.469	0.9846

*P value in the Cox proportional hazard model.

believe that this study can be helpful in daily clinical practice and in the decision-making process of thoracic surgeons when we encounter lung cancer patients with a history of surgery for colorectal cancer. We believe that our findings address an important issue regarding the clinical trials of lung cancers. In the future, further study is warranted regarding lung cancer patients with any extrapulmonary malignancies to evaluate the clinical behavior and survival of these patients in a prospective setting.

In conclusion, as a result of long-term survival and sufficient follow-up, the incidence of lung cancer in patients with a history of colorectal cancer has changed dramatically over time. Thus, a history of antecedent colorectal cancer did not contribute to the prognosis in patients with subsequent lung cancers. In the future, a multidisciplinary team management approach may be essential for developing customized treatment strategies in patients with lung cancer associated with antecedent colorectal cancer.

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Conflict of interest statement

None declared.

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ORIGINAL ARTICLE

THORACIC

Surgical outcomes of non-small-cell lung carcinoma in patients previously treated for gastric cancer

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Abstract

OBJECTIVES: Although the incidence of non-small-cell lung cancer (NSCLC) as a second malignancy is increasing, the prognosis remains controversial. Therefore, the present study aimed to determine the prognosis of patients with NSCLC who had previously been treated for gastric cancer (PGC).

METHODS: The clinicopathological records of patients who underwent complete surgical resection for NSCLC in three institutions from 2000 to 2013 were retrospectively investigated.

RESULTS: A total of 4651 patients were eligible for this study: 100 (2.1%) were patients with PGC and 4551 (97.9%) were patients with NSCLC who had not previously been treated for gastric cancer (NGC). The populations of older patients ($P < 0.001$), males ($P < 0.001$), limited resection for NSCLC ($P = 0.015$) and non-adenocarcinoma ($P = 0.024$) were significantly higher in the PGC, than in the NGC group. Overall survival did not significantly differ between the PGC and NGC groups (76.4 vs 74.5% $P = 0.82$). Multivariate analysis revealed that more advanced age, male sex, higher serum carcinoembryonic antigen levels, more advanced clinical stage of lung cancer and nonadenocarcinoma were independent factors for a poor prognosis, whereas a history of gastric cancer was not. None of the factors associated with gastric cancer affected the survival of patients with PGC.

CONCLUSIONS: After surgical treatment for lung cancer, a history of gastric cancer treatment had low impact on survival and no factors related to gastric cancer influence the outcomes. Curative surgery for NSCLC should be recommended when previously treated gastric cancer is well controlled.

Keywords: Gastric cancer • Non-small-cell lung cancer • Prognosis • Second primary tumours

INTRODUCTION

The incidence of non-small-cell lung cancer (NSCLC) as a second malignancy is increasing [1–3] because the survival of patients with many other types of malignancy has improved. Treatment for NSCLC as a second malignancy was determined considering the general status and the type or prognosis of previous cancer. However, whether surgical resection was the appropriate treatment for these patients is unclear because previous cancer might have affected the surgical outcomes of lung cancer. Although the prognosis of NSCLC as a second primary malignancy has been studied in detail [1–8], it remains controversial. One possible reason for this is population heterogeneity in the previous studies due to a variety of previous malignancies. Little survival data are available about patients with NSCLC and prior specific malignancies have been reported. We highlighted gastric cancer because it is a common malignancy [9] and it could affect the survival rates of lung cancer because it is associated with higher fatality rates [10]. The incidence rate of a second primary tumour developing after gastric cancer is 4.2% and such second primary tumours comprise lung cancer in 28.4% of these patients [11]. A few studies [1, 11] have addressed the clinical behaviour and survival of small cohorts of patients with NSCLC who had previously been treated for gastric cancer (PGC). Thus, the clinicopathological characteristics and the prognosis of patients with PGC remain unknown.

Here, we evaluated the clinicopathological features of PGC, assessed the prognosis of PGC and determined whether a history of gastric cancer influences the prognosis of NSCLC.

MATERIALS AND METHODS

Patient population

This study included 4782 patients who underwent complete surgical NSCLC resection at Hiroshima University Hospital (Hiroshima,

Japan), the National Cancer Center Hospital East (Chiba, Japan) and Juntendo University Hospital (Tokyo, Japan) between January 2000 and March 2013. We reviewed medical records and obtained clinicopathological information about gastric cancer and lung cancer for each patient. The surgical indications for primary lung cancer were discussed by each institutional cancer board. Sublobar resection was performed in cases of complete resection of the disease with appropriate surgical margins for small peripheral tumours. If lymph node metastasis was confirmed on an intraoperative frozen section of any lymph node, the procedure was converted to standard lobectomy. All stages were reclassified according the TNM classification of Malignant Tumors, 7th Edition [12]. The Institutional Review Boards at the participating hospitals approved the study and waived the requirement for the provision of written informed consent by individual patients.

Patients with NSCLC were assigned to groups according to whether they had been previously treated for gastric cancer (PGC) or not (NGC). Patients were excluded if they had incompletely resected NSCLC, missing information about treatment dates or stage of gastric cancer, stage IV gastric cancer and/or gastric cancer that was detected after surgery for lung cancer (Fig. 1). Data from the remaining 4651 patients were retrospectively analysed.

Pathological studies

Sections were fixed with 10% formalin and embedded in paraffin. Consecutive 4-μm sections were pathologically assessed using microscopy. Histological type was determined by staining with haematoxylin–eosin (H-E) and if the findings were inconclusive, the sections were immunohistochemically stained. Whether tumours were histologically second primary tumours or metastases was determined by pathologists from each institution based on immunohistochemical staining for TTF-1, CK7, CK20 SPA or Napsin A.

Statistical analysis

Data were statistically analysed using EZR (Saitama Medical Centre, Jichi Medical University; Kanda, 2012), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). Summarized data are presented as numbers or as means ± standard deviation unless otherwise stated. Categorical and continuous variables were compared using the χ^2 test and an unpaired *t*-test, respectively. Overall survival (OS) was defined as the interval between the date of lung surgery and that of death or the last follow-up visit. OS curves were calculated using the Kaplan–Meier method and survival differences among patients were assessed using the log-rank test. OS was assessed by univariate and multivariate analyses using the Cox proportional hazards model. A *P*-value of <0.05 was considered statistically significant.

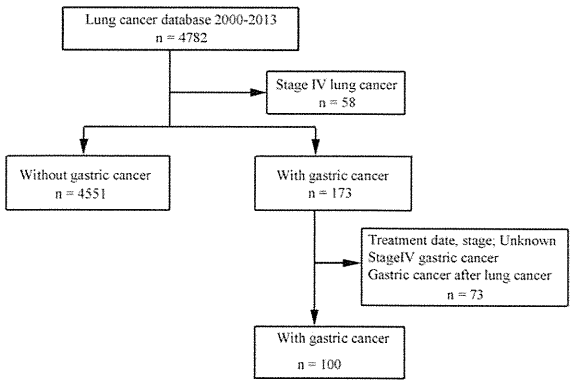

Figure 1: Flow of study cohort.

RESULTS

Clinical outcomes of patients with non-small-cell lung cancer who had previously been treated for gastric cancer

We assigned 100 (2.1%) and 4551 (97.9%) patients into PGC and NGC groups, respectively. The median follow-up duration was 62.3 months. A total of 952 patients died (PGC, $n = 16$; NGC, $n = 936$). The proportions of patients who were older (70.9 ± 7.6 vs 66.2 ± 9.7%, $P < 0.001$) and male (85 vs 61%, $P < 0.001$), and had limited resection of NSCLC (24 vs 15%, $P = 0.015$) and nonadenocarcinoma were significantly higher (39 vs 28%, $P = 0.024$) in the PGC, than in the NGC group. Serum CEA levels, clinical and pathological stages of lung cancer and adjuvant therapy for lung cancer did not significantly differ between the groups (Table 1).

Most patients in the PGC group had early-stage gastric cancer (78% had Stage I), and had been surgically treated for gastric cancer (73%). Only 3 patients had recurrent gastric cancer. Sixteen (16%) patients died (lung cancer, $n = 1$; gastric cancer, $n = 9$; other diseases, $n = 6$; Table 2). The median interval between gastric cancer and lung cancer was 3.2 (range 0–21.1) years. Thirty-two (41%) patients had undergone surgical lung cancer resection within 2 years of treatment for Stage I gastric cancer, and 31 (40%) had undergone such surgery over 5 years after treatment for gastric cancer (Fig. 2A). On the other hand, 7 (32%) and 8 (36%) patients underwent surgical resection for lung cancer within 2 and over 5 years after treatment for Stage II + III gastric cancer (Fig. 2B).

Prognosis of patients with non-small-cell lung cancer according to a history of treatment for gastric cancer

The 5-year OS rates did not significantly differ between the PGC and NGC groups (76.4 vs 74.5%, $P = 0.82$; Fig. 3), or between those in the two groups with Stages I and II + III gastric cancer (74.4 vs 74.5%, $P = 0.83$ and 83.0 vs 74.5%, $P = 0.93$). Univariate analysis of predictive factors for OS did not uncover a significant association with a history of gastric cancer (Table 3). Multivariate Cox analysis identified more advanced age, male sex, higher serum CEA levels and a higher clinical stage (II + III) of NSCLC as independent prognostic factors for poor OS, but not a history of gastric cancer [hazard ratio (HR) 1.17; 95% confidence interval (CI) 0.71–1.92; $P = 0.528$] (Table 3). We also examined predictive factors for OS in patients with PGC. Multivariate analysis did not associate OS with any of the factors related to gastric cancer, namely, pathological stage, method of treatment, adjuvant therapy and interval between gastric cancer and lung cancer (Table 4). Furthermore, OS did not significantly differ among intervals between gastric cancer and lung cancer (Table 5).

Table 1: Patients' characteristics

	All, $n = 4651$	PGC, $n = 100$ (%)	NGC, $n = 4551$ (%)	*P*-value
Age (years), means ± SD	66.3 ± 9.7	70.9 ± 7.6	66.2 ± 9.7	<0.001
Sex				
Male	2880	85 (85)	2795 (61)	<0.001
Female	1771	15 (15)	1756 (39)	
CEA (ng/ml), means ± SD	8.9 ± 65.1	9.6 ± 28.6	8.9 ± 65.7	0.912
cStage of lung cancer				
I	3678	80 (80)	3598 (79)	0.294
II	614	16 (16)	598 (13)	
III	359	4 (4)	355 (8)	
Surgical procedure				
Limited	694	24 (24)	670 (15)	0.015
Standard	3957	76 (76)	3881 (85)	
Histology				
Adenocarcinoma	3325	61 (61)	3264 (72)	0.024
Nonadenocarcinoma	1326	39 (39)	1287 (28)	
pStage of lung cancer				
I	3195	70 (70)	3125 (69)	0.725
II	711	17 (17)	694 (15)	
III	745	13 (13)	732 (16)	
Adjuvant therapy for lung cancer				
Yes	902	12 (12)	661 (15)	0.566
No	749	88 (88)	3890 (85)	

CEA: carcinoembryonic antigen; cStage: clinical stage; NGC: NSCLC who had not previously been treated for gastric cancer; PGC: NSCLC who had previously been treated for gastric cancer; pStage: pathological stage; SD: standard deviation.

Table 2: Clinicopathological characteristics of patients with previous gastric cancer

Factors	$n = 100$
pStage of gastric cancer	
I	78
II	13
III	9
Treatment methods for gastric cancer	
Endoscopic	27
Surgery	73
Interval between gastric cancer and lung cancer (years)	
≤5	61
>5	39
Adjuvant therapy for gastric cancer	
Yes	15
No	81
Recurrent gastric cancer	
Yes	3
No	97
Cause of death	
Lung cancer	9
Gastric cancer	1
Other	6

pStage: pathological stage.

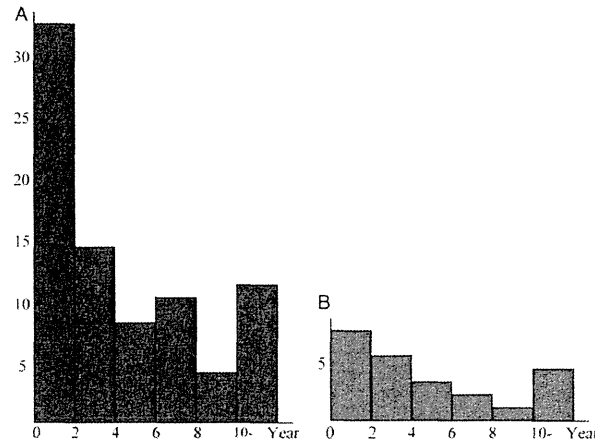

Figure 2: Number of patients based on interval from gastric cancer to lung cancer in the PGC group. Stage I (**A**) and Stage II/III (**B**) gastric cancer ($n = 78$ and $n = 22$, respectively). PGC: NSCLC who had previously been treated for gastric cancer.

DISCUSSION

The present study found a higher ratio of older and male patients in the PGC, than in the NGC group and similar prognoses between the two groups after complete surgical resection of lung cancer (5-year OS: 76.4 vs 74.5%; $P = 0.82$). Furthermore, multivariate analysis showed that a history of gastric cancer had low impact on the prognosis of patients after complete NSCLC resection.

Very few reports have described the survival of patients with PGC [1, 11]. Ikeda *et al.* [11] have shown that the prognosis of patients with gastric cancer and a second primary cancer is more negatively influenced by the second primary tumour than by the primary gastric cancer. Our findings were consistent with these results. Although lung cancer accounted for a high ratio of second primary cancers among patients with gastric cancer, the study by Ikeda *et al.* included many types of malignancies such as colorectal, liver and oesophageal cancers, as well as other malignancies including lung cancers. On the other hand, Pages *et al.* [1]

described particularly low OS rates among patients with NSCLC and a history of upper gastrointestinal malignancies. However, these studies included small sample cohorts and the prognosis of NSCLC with previous gastric cancer has remained controversial.

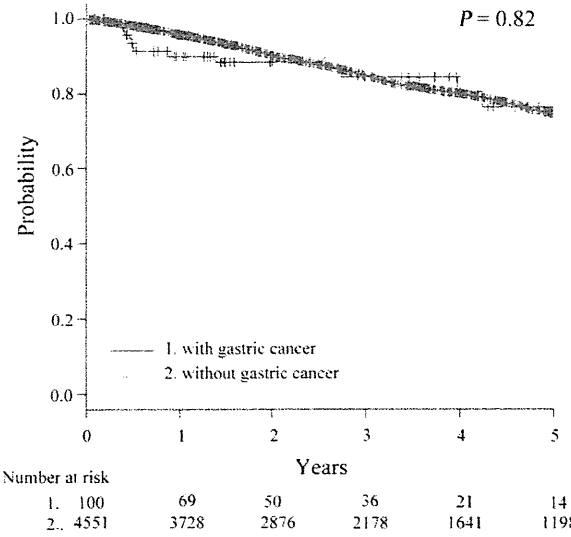

Figure 3: Kaplan–Meier overall survival curves according to history of gastric cancer. Five-year OS do not significantly differ between PGC and NGC (76.4 vs 74.0%, $P = 0.82$). OS: overall survival; PGC: NSCLC who had previously been treated for gastric cancer; NGC: NSCLC who had not previously been treated for gastric cancer.

The present multivariate analysis found that having had gastric cancer had low impact on the survival of patients with NSCLC when gastric cancer was curatively treated. Our multivariate analysis showed that patients without previous gastric cancer had slightly poorer outcomes than patients with previous gastric cancer (HR 1.17; without versus with previous gastric cancer), for unknown reasons. Variables that were not included in the analysis might have been involved. In addition, multivariate analysis did not uncover any factors related to gastric cancer that were associated with OS in the PGC group and the findings of multivariate analysis were similar in both the PGC and the NGC groups (data not shown). Thus, if gastric cancer was considered controlled, then it appeared not to influence prognosis after surgical resection of lung cancer. Although the criteria for controllable gastric cancer were not defined herein, Stage I gastric cancer was considered controllable. The reason is that OS did not significantly differ between patients in the PGC group with Stage I gastric cancer and the NGC group. The results were similar for Stage II + III gastric cancer, but these findings should be carefully considered in light of the following. Firstly, only 22 patients had Stage II + III gastric cancer. Since survival rates were worse for patients with advanced than early-stage gastric cancer, a second cancer might occur less frequently in those with Stage II + III than Stage I gastric cancer because the follow-up term is short. Secondly, about 40% of surgical resections to treat lung cancer in the PGC group with Stage I gastric cancer were performed within 2 years after gastric cancer treatment. On the other hand, surgical resection to treat lung cancer in the PGC group with Stage II + III gastric cancer might be avoided even if lung cancer is diagnosed at the early stage after gastric cancer treatment.

Table 3: Univariate and multivariate Cox regression analysis of OS of all patients

	Univariate			Multivariate		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age (≥75 vs <75 years)	1.79	1.55–2.07	<0.001	1.79	1.54–2.07	<0.001
Sex (male versus female)	2.17	1.86–2.52	<0.001	1.56	1.33–1.84	<0.001
CEA (>5.0 vs ≤5.0 ng/ml)	2.10	1.85–2.39	<0.001	1.71	1.50–1.95	<0.001
cStage of lung cancer (II + III versus I)	3.90	3.42–4.44	<0.001	2.17	1.88–2.50	<0.001
History of gastric cancer (without versus with)	0.94	0.57–1.54	0.819	1.17	0.71–1.92	0.528
Lung cancer (non-ad versus ad)	2.04	1.79–2.31	<0.001	1.29	1.10–1.46	0.001
Adjuvant therapy for lung cancer (yes versus no)	1.16	0.95–1.42	0.134	1.13	0.92–1.39	0.233

Ad: adenocarcinoma; CI: confidence interval; cStage: clinical stage; HR: hazard ratio; OS: overall survival.

Table 4: Univariate and multivariate Cox regression analysis of OS of patients with previous gastric cancer

	Univariate			Multivariate		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
pStage of gastric cancer (II + III versus I)	0.92	0.26–3.27	0.901	0.75	0.17–3.32	0.708
Treatment for gastric cancer (endoscopic versus surgery)	0.97	0.33–2.82	0.961	1.16	0.35–3.86	0.802
Adjuvant therapy for gastric cancer (yes versus no)	2.90	0.74–11.36	0.124	3.47	0.70–17.15	0.125
Interval between gastric and lung cancer (≥5 vs <5 years)	0.93	0.36–2.54	0.900	0.93	0.31–2.77	0.904

CI: confidence interval; HR: hazard ratio; OS: overall survival; pStage: pathological stage.