RTKs [6,7]. The Eph receptors are divided into EphA and EphB on the basis of structural homology and ligand-binding affinity [8,9]. The ligands for the Eph receptors are ephrins, which are membrane-bound and also fall into 2 subclasses, ephrin-A and -B [10]. The Eph-ephrin system plays an important role in normal embryonic development processes, especially in neuronal development [8,10]. Among these Eph families, EphA2 is currently being extensively studied because of the accumulation of evidence, which supports a strong association with tumor development and progression. Such relationships with EphA2 have been reported in many cancers, including glioblastoma [11,12], colorectal [13], gastric [14], esophageal [15], breast [16], ovarian [17], endometrial [18], cervical [19], pancreatic [20], prostate [21], melanoma [22], bladder [23], renal cell [24], and hepatocellular carcinoma [25]. In addition, up-regulation of this molecule has been reported in NSCLC, indicating that higher expression is positively related to brain metastasis [26], smoking history [27], and poorer survival [28,29].

Although these findings are strong enough to render this molecule as one of the promising biomarkers or therapeutic targets for NSCLC, the complexity rests with the signaling pathway conducted by its ligand ephrin-A1; its signal is bi-directional, and activation of EphA2 by ephrin-A1 triggers signaling events that are more tumor-suppressive.

Due to the complexity of this interaction, greater clarification is required on the expression levels and the mechanisms of this system involved in each cancer type and stage. In this study, we conducted a retrospective study to evaluate EphA2 and ephrin-A1 expression levels in p-stage I NSCLC patients at both the genetic and protein levels, and analyzed their respective associated clinicopathologic features and clinical outcomes. By focusing on a patient population of surgically treated p-stage I NSCLC patients, it allowed us to reveal the possible divergent roles of EphA2 and ephrin-A1 in cancer which will provide us with useful information for targeting this system in treatment of NSCLC.

2. Materials and methods

2.1. Patients and tissue samples

Tissue samples were obtained from patients who had undergone complete surgical resection of p-stage I primary NSCLC without any prior anticancer therapies at Kyoto University Hospital from May 2001 through July 2005. The sample size was estimated to detect about 15% difference of five-year overall survival after dichotomization between two groups. p-Stage was determined by the latest tumor–node–metastasis classification system [30]. Histological type and grade of cell differentiation were determined according to the WHO classification system [31]. Informed consent for participation in this study was obtained from all patients prior to the surgical operations. This study was reviewed and approved by the Ethics Committee of the Graduate School and Faculty of Medicine at Kyoto University.

2.2. mRNA isolation and cDNA synthesis

For sample collection, tumor tissue samples were dissected, in their entirety, immediately after surgical resection and soaked in RNAlater TissueProtect Tubes (Qiagen Sciences, Tokyo, Japan) for more than 2 days and then stored at −80 °C until use. Total mRNA was isolated from tissue samples using RNeasy Plus Mini Kit (Qiagen Sciences) and reverse transcription of total mRNA was conducted using the Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences, Uppsala, Sweden) to obtain total cDNA.

2.3. Quantification of EphA2/ephrin-A1 mRNA

To quantify EphA2/ephrin-A1 mRNA expression level of each sample, quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the LightCycler thermal cycler system (Roche Diagnostics Japan, Tokyo, Japan). The PCR primers used for the quantitative amplification of EphA2 were forward: 5′-GTGTACAAGGGCATGCTGAA-3′ and reverse: 5′-AACTTGTCCAGGGCCCCATT-3′, which amplified a 230-base pair fragment of EphA2 cDNA. The primers for the quantitative amplification of ephrin-A1 were forward: 5′-AACAAGCTGTGCAGGCATGG-3′ and reverse: 5′-CTCCACAGATGAGGTCTTGC-3′, which amplified a 230-base pair fragment of ephrin-A1 cDNA. The primers for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, used as an internal control, were forward: 5′-ACAACAGCCTCAAGATCATCAG-3′ and reverse: 5′-TCTTCTGGGTGGCAGTGATG-3′. A 20-μL reaction mixture containing 0.5 μM forward and reverse primers and 0.03 μg cDNA in QuantiTect SYBR Green PCR Master Mix (Qiagen Sciences) was prepared. PCR amplification was initiated by preincubation for 15 minutes at 95 °C for initial activation, followed by 35 cycles of the following protocol: denaturation at 94 °C for 15 seconds (s), annealing at 59 °C for 15 s, and elongation at 72 °C for 15 s with detection of fluorescence products. The quantitative data were analyzed with LightCycler analysis software version 5.03. The expression level of EphA2/ephrin-A1 was represented as the ratio (%) of EphA2/ephrin-A1 mRNA value to GAPDH mRNA.

2.4. Immunohistochemical study

Immunohistochemical staining was performed using Dako LSAB+System-HRP (Dako Japan, Tokyo, Japan). Formalin-fixed paraffin-embedded tissue was cut into 4 μm sections and mounted on glass slides. After deparaffinization and rehydration, the slides were heated in a buffer solution (HistoVT One, Nacalai Tesque, Kyoto, Japan) for antigen retrieval at the temperature of 90 °C for 20 minutes (min). After quenching the endogenous activity with 0.3% hydrogen peroxide (in absolute methanol) for 10 min, the sections were treated with blocking agents (DAKOCytomation Protein Block, Dako Japan) for 30 min to block nonspecific staining. The sections were incubated overnight with rabbit anti-EphA2 (sc-924, 1:100) and anti-ephrin-A1 (sc-911, 1:100) antibodies (both from Santa Cruz Biotechnology Inc., CA, USA). The slides were incubated for 50 min with the secondary antibody (Biotinylated Link, Dako Japan), incubated with peroxidase (STREPTOAVIDIN-HRP, Dako Japan) for 50 min, and the antibody binding was visualized with 3,3′-diaminobenzine tetrahydrochloride (DAB+ CHROMOGEN, Dako Japan). Finally, the sections were counterstained with Mayer's hematoxylin (Dako REAL Hematoxylin, Dako Japan). Human NSCLC specimens that had already been shown to express EphA2 and ephrin-A1 were included in all series as positive controls. The negative control slides were prepared by replacing the primary antibody with antigen diluent only.

2.5. Detection of the EGFR and K-ras gene mutations

The EGFR gene mutations (exons 18–21) were detected using the PCR-single strand conformational polymorphism analysis, and K-ras gene mutations (codon 12) were screened using the mutagenic PCR-restriction enzyme fragment length polymorphism method [32].

2.6. Evaluation of mRNA expression

The patients were divided into EphA2-Low and EphA2-High groups on the basis of the median EphA2 mRNA expression level,

and their clinicopathologic features and overall/disease-free survival curves were analyzed. The same analysis was conducted regarding ephrin-A1 mRNA expression levels (ephrin-A1-Low and ephrin-A1-High groups).

2.7. Evaluation for immunohistochemical study

The EphA2/ephrin-A1 expressions were estimated according to the staining intensities of the cancer cells and graded as negative, weak, moderate, and strong staining. Weak staining was defined as clear staining at the same level as observed in the internal controls (normal vascular endothelial cells). The slides were reviewed independently by two investigators (M. I. and R. M.) without knowledge of clinical data and differences were later resolved by consensus. Since tumor cells were stained almost uniformly across the samples, we did not consider the fraction of tumor cells at each staining level. The patients were divided into two groups according to their staining intensity (EphA2/ephrin-A1 -IHC-Low and EphA2/ephrin-A1 -IHC-High groups), and the respective clinicopathological features and overall/disease-free survival curves were analyzed.

2.8. Statistical analysis

Statistically significant differences within each categorical data were determined using the chi-squared test. Continuous data were compared using Student's *t*-test. The postoperative survival and disease-free survival rates were calculated using the Kaplan–Meier method and compared using the log-rank test. Probability values of $P < 0.05$ were considered significant. All statistical analyses were performed using StatMate IV software version 4.01 (ATMS, Tokyo, Japan) and JMP software version 8 (SAS Institute Japan, Tokyo, Japan).

3. Results

3.1. EphA2 and ephrin-A1 mRNA expression levels in p-stage I NSCLC patients

A total of 195 patients were enrolled in the study; their demographics and baseline characteristics are presented in Table 1. EphA2 mRNA was detected in p-stage I NSCLC tissue samples from all 195 patients, and ranged from 0.0083% to 21.1%. The median expression level was approximately 1.7% and, based on this median, 98 patients were placed in the EphA2-Low group and 97 in the EphA2-High group. Due to a shortage of samples, only 191 patients could be classified according to ephrin-A1 status; the levels of ephrin-A1 mRNA detected in these 191 samples ranged from 0.0009% to 62.6%. The median level was approximately 0.6% and, based on this median, 96 patients were placed in the ephrin-A1-Low group and 95 in the ephrin-A1-High group.

The relationships of the EphA2 and ephrin-A1 mRNA expression status to clinicopathologic factors are shown in Table 2. There were statistically significant differences between the EphA2-Low and EphA2-High groups with respect to sex, smoking status (including pack-years), histology, tumor differentiation, pathologic stage, and EGFR gene mutations. All these differences were also detected between the ephrin-A1-Low and ephrin-A1-High groups. In terms of age, operation method, adjuvant chemotherapy, and K-ras mutation, there were no statistical differences in either molecular category.

Among the four groups created by combining these two molecular categories (i.e., EphA2/ephrin-A1; -Low/-Low, -Low/-High, -High/-Low, -High/-High), a higher number of cases were classified in the EphA2/ephrin-A1 -Low/-Low group and -High/-High group ($n = 64$ and 64, respectively) compared to the EphA2-Low/ephrin-A1-High group and the EphA2-High/ephrin-A1-Low group ($n = 31$ and 32, respectively) ($P < 0.001$, chi-squared test).

Table 1
Characteristics of the patients included in the study.

	Numbers (*n*)	Percent (%)
Age (years)		
Mean ± SD (range)	66.5	±10.4 (23–88)
Sex		
Male	114	58.5
Female	81	41.5
Smoking status		
Never smoker	72	36.9
Ex-smoker	50	25.6
Current smoker	73	37.4
Pack-year/mean ± SD (range)	33.1	±39.3 (0–250)
Histology		
Adenocarcinoma	142	72.8
Squamous cell carcinoma	42	21.5
Adenosquamous cell carcinoma	2	1
Large cell carcinoma	5	2.6
LCNEC	2	1
Pleomorphic carcinoma	2	1
Tumor differentiation		
Well	82	42.1
Moderately	84	43.1
Poorly	29	14.9
Pathological stage		
IA	129	66.2
IB	66	33.8
Operation method		
Lobectomy	140	71.8
Segmentectomy	37	19
Partial resection	18	9.2
Adjuvat chemotherapy		
No	60	30.8
UFT	126	64.6
I.V. (with or without UFT)	9	4.6
EGFR mutation status		
Mutation (+)	80	41
Wild type	115	59
K-ras mutation status		
Mutation (+)	18	9.2
Wild type	177	90.8

SD: standard deviation; LCNEC: large cell neuroendocrine carcinoma; I.V.: intravenously administered chemotherapeutic agents.

3.2. Postoperative overall survival and disease-free survival

Five-year survival rates in the EphA2-Low and EphA2-High groups were 68.9% and 86.1%, respectively. Log-rank testing revealed the difference was statistically significant ($P = 0.017$; hazard ratio (HR) = 2.11, with a 95% confidence interval (CI) of 1.14–3.90). Five-year disease-free survival rates in the EphA2-Low and EphA2-High groups were 69.9% and 83.2%, respectively, and Log-rank testing also revealed a statistical difference ($P = 0.035$; HR = 1.94, with a 95% CI of 1.04–3.60)). Kaplan–Meier survival curves are shown in Fig. 1A.

Five-year survival rates in the ephrin-A1-Low and ephrin-A1-High groups were 71.8% and 82.4%, respectively, and five-year disease-free survival rates were 71.9% and 80.2%, respectively. There were no statistical differences between these groups ($P = 0.16$ and 0.24, respectively). Kaplan–Meier survival curves are shown in Fig. 1B.

3.3. Immunohistochemical staining study

Of the 195 samples, 183 were available for staining; the other samples were non-evaluable. The numbers of tumor samples classified as EphA2-negative, -weak, -moderate, and -strong were 5, 50, 85, and 43, respectively, and the numbers of ephrin-A1-negative,

Table 2
EphA2 and ephrin-A1 mRNA expression and characteristics of the patients.

	EphA2	(n=195)		Ephrin-A1	(n=191)	
	Low (n=98)	High (n=97)	P-value[a]	Low (n=96)	High (n=95)	P-value[a]
Age (years)						
Mean ± SD	67.1 ± 10.6	66.0 ± 10.2	0.44	67.0 ± 10.3	66.4 ± 10.4	0.65
Sex						
Male	68	46	0.002	64	48	0.024
Female	30	51		32	47	
Smoking status						
Never smoked	23	49	<0.001	26	43	0.01
Ex-smoker	26	24		24	25	
Current smoker	49	24		46	27	
Pack-year/mean ± SD	43.5 ± 36.6	22.7 ± 39.2	<0.001	41.8 ± 41.7	25.0 ± 35.1	0.003
Histology						
Adenocarcinoma	53	89	<0.001	55	84	<0.001
Squamous cell carcinoma	35	7		33	8	
Adenosquamous cell carcinoma	2	0		1	1	
Large cell carcinoma	5	0		4	1	
LCNEC	2	0		2	0	
Pleomorphic carcinoma	1	1		1	1	
Tumor differentiation						
Well	21	61	<0.001	30	51	0.004
Moderately	50	34		46	35	
Poorly	27	2		20	9	
Pathological stage						
IA	56	73	0.008	57	70	0.036
IB	42	24		39	25	
Operation method						
Lobectomy	67	73	0.45	62	75	0.066
Segmentectomy	22	15		24	12	
Partial resection	9	9		10	8	
Adjuvat chemotherapy						
No	33	27	0.61	29	30	0.91
UFT	60	66		63	60	
I.V. (with or without UFT)	5	4		4	5	
EGFR mutation status						
Mutation (+)	22	58	<0.001	24	54	<0.001
Wild type	76	39		72	41	
K-ras mutation status						
Mutation (+)	8	10	0.6	13	5	0.05
Wild type	90	87		83	90	

[a] Calculated by chi-squared test except for patients' age and smoking pack-year (Student's t-test).

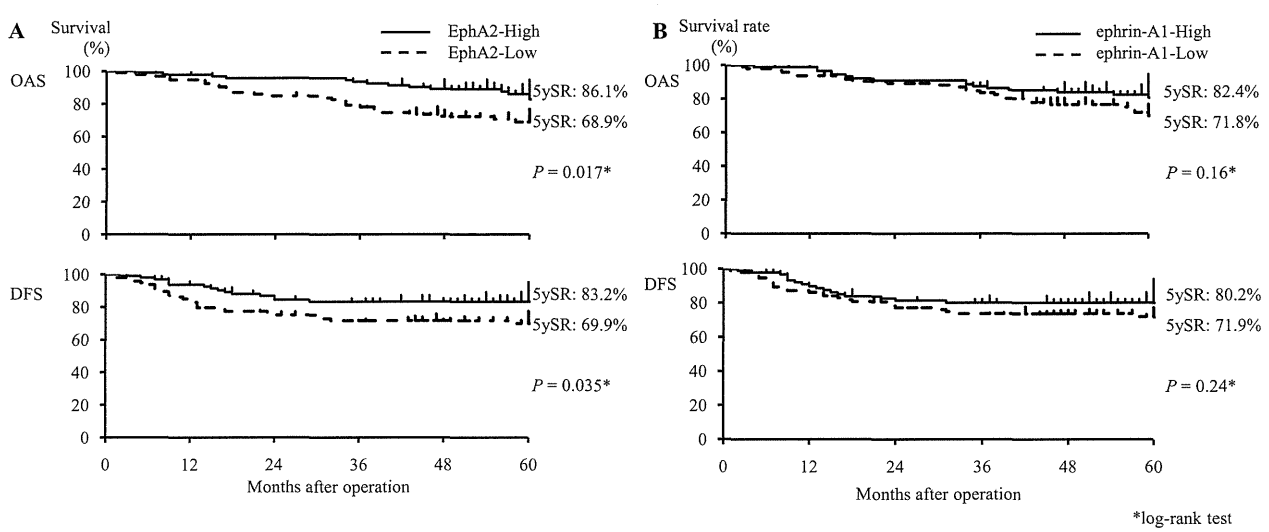

Fig. 1. Postoperative survival curves of p-stage I NSCLC patients (top: overall survival (OAS), bottom: disease-free survival (DFS)), compared according to the EphA2 (A) and the ephrin-A1 (B) mRNA expression levels. 5ySR, five-year survival rate, P-values were calculated by the log-rank test.

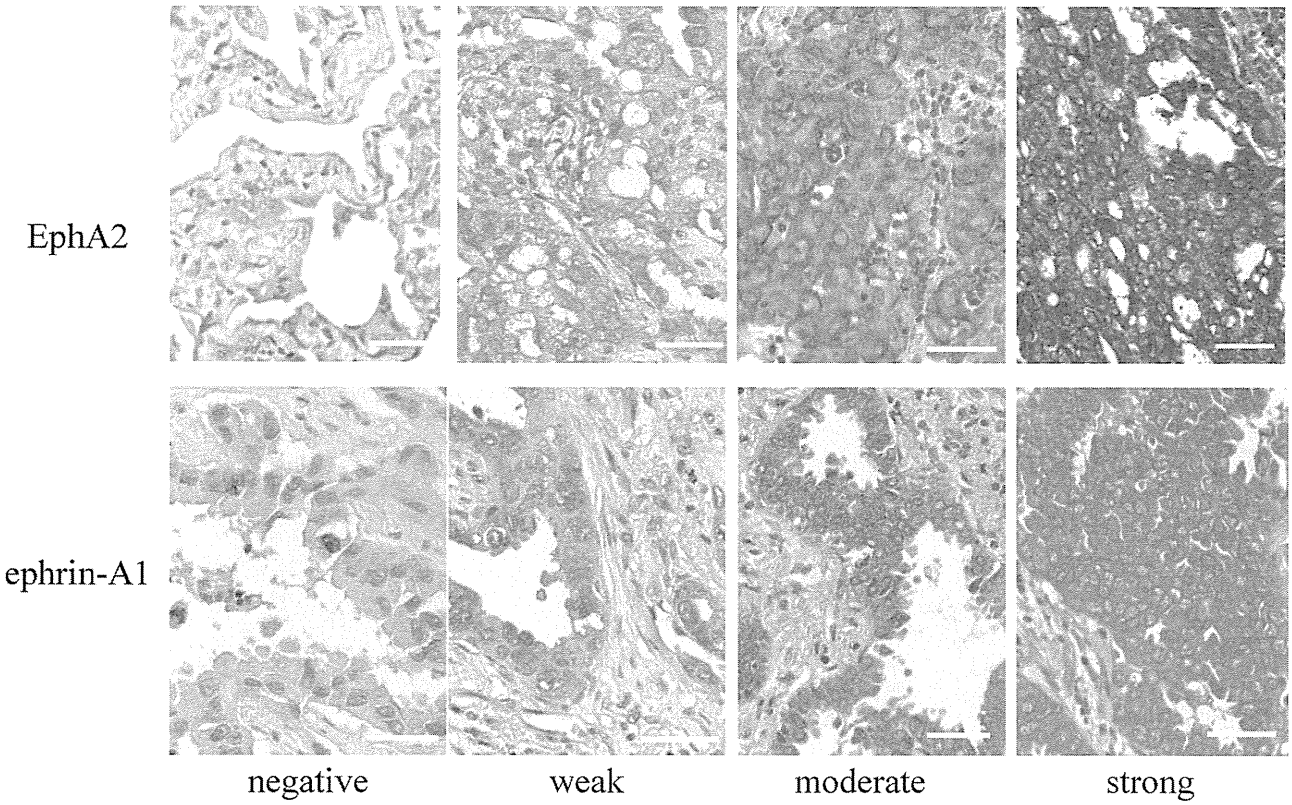

Fig. 2. Immunohistochemical staining for EphA2 and ephrin-A1. Scale bar, 40 μm.

-weak, -moderate, and -strong were 9, 44, 93, and 37, respectively (Fig. 2). As little difference was found between weak and moderate staining, analyses were conducted by grouping them into EphA2/ephrin-A1 -IHC-Low (negative/weak/moderate staining) and -IHC-High (strong staining) groups. The comparative demographics of these groups are presented in Table 3.

Statistically significant differences were found only in sex, smoking (pack-years), histology, and EGFR mutation between the EphA2-IHC-Low and EphA2-IHC-High groups, and only in histology and tumor differentiation (adenocarcinoma vs. non-adenocarcinoma) between the ephrin-A1-IHC-Low and ephrin-A1-IHC-High groups. In this four-category grouping by mRNA expression levels, the distribution pattern was statistically significant with the EphA2/ephrin-A1-IHC-Low and -IHC-High groups having a larger number of cases ($n=117$ and 15, respectively) than the EphA2-IHC-Low/ephrin-A1-IHC-High and EphA2-IHC-High/ephrin-A1-IHC-Low groups ($n=22$ and 28, respectively) ($P=0.007$, chi-squared test).

No correlation was seen between the mRNA expression level and immunohistochemical staining levels.

3.4. Clinical outcomes of immunohistochemical staining study

Five-year survival rates in the EphA2-IHC-Low and EphA2-IHC-High groups were 75.0% and 88.0%, respectively, and five-year disease-free survival rates were 74.7% and 85.5%, respectively. No statistical differences were found between these groups ($P=0.13$ and 0.23, respectively). Kaplan–Meier survival curves are shown in Fig. 3A.

Similarly, five-year survival rates in the ephrin-A1-IHC-Low and the ephrin-A1-IHC-High groups were 76.1% and 87.9%, respectively, and five-year disease-free survival rates were 74.6% and 88.0%, respectively. No statistical differences were detected ($P=0.12$ and 0.13, respectively). Kaplan–Meier survival curves are shown in Fig. 3B.

4. Discussion

As for NSCLC, Kinch et al. [26] previously reported that EphA2 immunohistochemical staining intensity differs according to NSCLC stages and primary/metastasis sites, and Brannan et al. [27] reported that higher EphA2 immunohistochemical expression was correlated with poorer prognosis. Faoro et al. [29] revealed the histological differences in EphA2 immunohistochemical staining levels. In general, up-regulation of this molecule is related to poorer clinical outcomes in many types of cancer patients.

However, recent interest has been focused on its conflicting role in tumor suppressive activity triggered by its ligand, ephrin-A1. One hypothesis is that while the EphA2/ephrin-A1 system itself suppresses oncogenic pathways, the lack of ephrin-A1 leads to an accumulation of nonphosphorylated EphA2, resulting in an oncogenic system [9]. Recently, Miao et al. [33] reported that the ligand-dependent inhibition and ligand-independent promotion of cell migration is mediated by EphA2.

Here, our research showed that the EphA2-High and ephrin-A1-High group patients shared favorable prognostic factors in p-stage I NSCLC. It is speculated that tumors of patients with these clinicopathologic features express higher levels of EphA2 and ephrin-A1, and thereby, supposedly exert their tumor suppressive function. In addition, the positive relationship we found between the expression levels of these two molecules suggests the existence of some positive feedback mechanism between receptor and ligand. Despite showing a smaller difference, the immunohistochemical staining results also supported these observations.

Table 3
EphA2 and ephrin-A1 immunohistochemical staining intensity and characteristics of the patients.

	EphA2-IHC ($n=183$)			Ephrin-A1-IHC ($n=183$)		
	Low ($n=140$)	High ($n=43$)	P-value[a]	Low ($n=146$)	High ($n=37$)	P-value[a]
Age (years)						
Mean ± SD	66.1 ± 10.5	68.4 ± 10.2	0.2	66.8 ± 10.9	65.7 ± 10.4	0.57
Sex						
Male	86	19	0.046	86	18	0.26
Female	54	24		60	19	
Smoking status						
Never smoked	57	22	0.24	53	17	0.53
Ex-smoker	35	12		38	9	
Current smoker	48	9		55	11	
Pack-year/mean ± SD	33.9 ± 35.7	19.2 ± 31.4	0.016	32.8 ± 35.1	21.4 ± 34.8	0.08
Histology						
Adenocarcinoma	97	42	0.008	104	35	0.11
Squamous cell carcinoma	37	0		35	2	
Adenosquamous cell carcinoma	1	0		1	0	
Large cell carcinoma	4	1		5	0	(Ad/non-Ad) 0.003
LCNEC	1	0		1	0	
Pleomorphic carcinoma	0	0		0	0	
Tumor differentiation						
Well	56	23	0.26	56	23	0.023
Moderately	62	16		66	12	
Poorly	22	4		24	2	
Pathological stage						
IA	94	27	0.6	94	27	0.32
IB	46	16		52	10	
Operation method						
Lobectomy	103	29	0.72	106	26	0.93
Segmentectomy	25	9		27	7	
Partial resection	12	5		13	4	
Adjuvat chemotherapy						
No	42	14	0.28	40	16	0.14
UFT	90	29		100	19	
I.V. (with or without UFT)	8	0		6	2	
EGFR mutation status						
Mutation (+)	52	25	0.015	58	19	0.2
Wild type	88	18		88	18	
K-ras mutation status						
Mutation (+)	14	3	0.55	15	2	0.36
Wild type	126	40		131	35	

[a] Calculated by chi-squared test except for patients' age and smoking pack-year (Student's t-test).

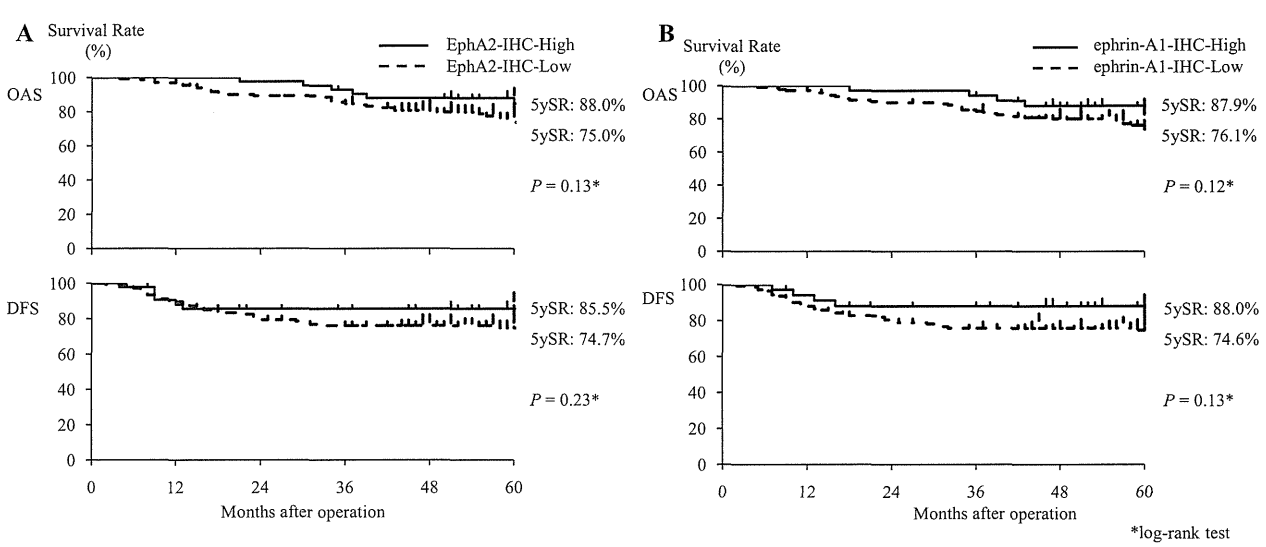

Fig. 3. Postoperative survival curves of p-stage I NSCLC patients (top: overall survival (OAS), bottom: disease-free survival (DFS)), compared according to the EphA2 (A) and the ephrin-A1 (B) immunohistochemical staining levels. 5ySR, five-year survival rate, P-values were calculated by the log-rank test.

Although specific reasons for the discrepancies between genetic expression (qRT-PCR) and protein expression (immunohistochemical staining) in the clinical samples could not be identified here, some possible explanations include: the existence of some regulatory steps in the course of mRNA translation; the process of IHC is more easily influenced by small variations in the experimental conditions (i.e., temperature, time, specimens preservation) than qRT-PCR because they are not mechanically controlled; and the arbitrariness in evaluating IHC samples. Nevertheless, demonstration of the same distribution patterns here is intriguing and renders them more evidential.

In this manuscript, our genetic analysis was unique in that the study population was limited to well-defined type of patients with surgically treated p-stage I NSCLC, which allowed us to obtain the samples directly from the surgical site, and the groupings were done according to the median level observed within these patients. As seen in the previous study, EphA2 protein expression levels may increase according to the NSCLC stage [26], and analyzing all stages of NSCLC patients together (clinical stage I-IV) means shifting the cutoff line into higher levels, thereby worsening the prognosis of higher EphA2 expression patients. These arguments partly explain the inconsistency seen in our results.

Another contributory factor that may explain our results is the expression of ephrin-A1, which is generally considered to be associated with favorable prognosis. In this study, ephrin-A1 and EphA2 expression patterns were well correlated with each other. Previous reports on EphA2 expression in NSCLC did not provide any information on ephrin-A1 expression. We hope that further investigations might identify this phenomenon in other types of cancer.

Other Eph families have also been shown to be negatively associated with lung cancer. For example, EphA1, A3, A4, A5, A7, and B4 are some of the most common types of activated RTKs in human lung cancers [34]. Further, EphA3, along with A5, A7, B1, B6, were shown to be mutated in lung adenocarcinoma patients [35], and EphB3 was reported to be overexpressed and to promote tumorigenicity in NSCLC [36]. Inversely, decreased EphB6 expression was associated with an increased risk for metastasis in NSCLC patients [37]. These findings leave us much room for further investigations on the Eph families.

As a biomarker, EphA2 serves as a favorable prognostic marker in p-stage I NSCLC, as shown in our study. Our future direction is to establish a practical evaluation system of EphA2 expression and utilize it as a prognostic factor along with other clinicopathological features in p-stage I NSCLC.

As a target for cancer therapy, it is already reported that cytotoxic agents, antibodies, siRNAs, or vaccines targeting these molecules are candidates as anti-cancer agents [38–42]. However, tyrosine kinase inhibitors targeting EphA2 can be rather harmful for preventing cancer development, by suppressing the anti-oncogenic activities of the EphA2/ephrin-A1 system [33]. Furthermore, our results suggest that the therapeutic modalities targeting these molecules require the tumor stage or patients' backgrounds to be taken into account. We hope that this study will be helpful for facilitating the development of more efficient therapeutic modalities for NSCLC.

5. Conclusions

Our study suggested that, in p-stage I NSCLC patients, those in the higher EphA2 expression and higher ephrin-A1 expression groups shared almost the same clinicopathological backgrounds which are generally considered to be better prognostic factors, and that higher EphA2 mRNA expression is a better prognostic factor compared with ephrin-A1 mRNA expression, while the immunohistochemical study, although not contradictory, nevertheless did not identify any statistically significant differences in clinical outcomes.

These propensities have not been reported previously, and these results suggest the possibility that the EphA2/ephrin-A1 system plays a complex role in the development of NSCLC especially at an early stage. Further study will illuminate the complicated and biphasic roles of these molecules, making them much more promising biomarkers and therapeutic targets for NSCLC.

6. Conflict of interest statement

There were no conflicts of interest in this study.

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ORIGINAL ARTICLE

Current status of postoperative follow-up for lung cancer in Japan: questionnaire survey by the Setouchi Lung Cancer Study Group—A0901

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Abstract
Purpose. There is no recommended standard follow-up program after resection for lung cancer. Under these circumstances, each doctor establishes his or her own follow-up protocol. This questionnaire survey was conducted to grasp the current status of postoperative follow-up in Japan.
Methods. The questionnaire survey was aimed at determining what examinations were performed and at what frequencies in the setting of postoperative follow-up. Based on these results, examinations performed at a frequency of >50% and the time points after resection at which they were performed were selected and presented as components of an average follow-up program.
Results. Questionnaires were sent to 44 institutions, and 26 doctors responded to the questionnaire. All 26 of the doctors performed physical examinations, blood examinations, chest radiography, and computed tomography (CT) routinely, but their frequencies varied widely among the doctors. The average frequencies of the follow-up examinations as judged from this survey are as follows: Physical and blood examinations are performed three to four times a year for the first 3 years and twice a year during the next 2 years. CT is scheduled at 6 and 12 months after resection and is repeated annually thereafter. Chest radiography is performed three to four times a year for the first 3 years and once a year thereafter, between the CT examinations.
Conclusion. The follow-up programs used in clinical practice vary widely among institutions and doctors in terms of the types of examination performed and the frequencies at which they are performed.

Key words Lung cancer · Postoperative follow-up · Postoperative surveillance · Recurrence

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Introduction

Surgical resection with curative intent is selected for the treatment of localized non-small-cell lung cancer (NSCLC). The 5-year survival rate after complete resection for NSCLC is approximately 60%, and many patients develop recurrences after resection.[1] To detect recurrences, several examinations are performed periodically as part of the postoperative follow-up or surveillance. The purpose of postoperative follow-up is to detect recurrences and/or metachronous tumors, so adequate treatment can be offered in an attempt to improve the survival duration and the quality of life. Some investigators have suggested that the survival duration is greater in patients with asymptomatic recurrences detected by follow-up examinations than in those with symptomatic recurrences, and that follow-up examinations after resection are useful for detecting asymptomatic recurrences.[2,3] At the same time, several investigators have reported that the benefit of postoperative follow-up is questionable from the point of view of efficacy and cost-effectiveness.[4–8] Thus, the benefits and efficacy of postoperative follow-up remains controversial.

The board of the Japan Lung Cancer Society drew up a clinical practice guideline for lung cancer in 2005, and periodic follow-up after resection is not recommended in this guideline because there was still no clear persuasive evidence to support it.[9] Under these circumstances, each institution or each doctor establishes his or her own postoperative follow-up program in clinical practice. It is suspected that these postoperative follow-up protocols applied in clinical practice vary widely in terms of the examinations performed and their frequency. To grasp the current status of follow-up after resection for NSCLC in Japan, a questionnaire survey of the institutions affiliated with the Setouchi Lung Cancer Study Group was performed to determine the kinds of examination and the frequencies at which they are performed in the setting of postoperative follow-up. In addition, examinations that were performed frequently and the time points after resection at which they were performed were selected, and the average follow-up protocol based on the results of the questionnaire survey is presented.

Methods

A questionnaire designed to obtain information regarding the postoperative follow-up protocol adopted for NSCLC patients was sent by mail to 44 institutions affiliated with the Setouchi Lung Cancer Study Group. The questionnaire consisted of the following questions.

1. Is a standardized follow-up protocol followed at the institution?
2. Does the follow-up schedule differ depending on the disease stage?
3. What are the examination modalities chosen in the setting of postoperative follow-up? At what frequencies are these examinations performed? Please record your answers in Table 1.

Based on the information in this survey, the percentage of the 26 doctors who performed the examinations was calculated for each of the examinations at each time point after the resection. Then, examinations that were performed at a frequency of >50% and the time points after resection at which they were performed were selected and are presented as components of an average follow-up program in this study.

The TNM stage was determined according to the Union for International Cancer Control (UICC) TNM classification of pathological stage, 6th edition.[10]

Results

Questionnaires were sent to 44 institutions affiliated with the Setouchi Lung Cancer Study Group, 17 (38.6%) of which responded to the questionnaire. From these 17 institutions, 26 doctors, comprising 2 oncologists and 24 thoracic surgeons, responded to the questionnaire.

Of the 17 institutions, 7 reported that they followed a standardized institutional follow-up program, whereas the remaining 10 institutions did not (Table 2). Of the 26 doctors, 11 discontinued the follow-up 5 years after the resection, whereas the remaining 15 continued follow-up for >5 years after the resection. Among the 26 doctors, 15 arrange follow-up schedules based on the disease stage; for example, six doctors classified the patients into two groups based on the disease stage (stage IA and other stages), and four classified the patients into three groups based on the disease stage (IA, IB/II, and IIIA). Each of the 26 doctors performed blood examinations, chest radiography, and computed tomography (CT) routinely. Six doctors performed positron emission tomography (PET) or PET/CT, and nine doctors performed brain magnetic resonance imaging (MRI) or brain CT routinely. None of the doctors performed sputum cytology or abdominal ultrasonography (US) in the setting of postoperative follow-up.

Figure 1 shows the frequency of each of the examinations performed after the resection. The Y-axis shows the percentage of doctors who performed the examinations, and the X-axis shows the time of performance of the examination after the resection. More than half of

Table 1 Questionnaire

Procedure	1 Year					2 Years				3 Years				4 Years				5 Years				6 Years		7 Years		8 Years		9 Years		10 Years	
	1	2	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	6	12	6	12	6	12	6	12	6	12
Physical examination																																
Blood examination (tumor markers)																																
Sputum cytology																																
Chest radiography																																
Abdominal US																																
CT																																
Bone scintigraphy																																
Brain MRI/CT																																
PET/CT																																
Other examinations																																

The numbers underneath the 1–10 years are the months (1, 2, 3, 6, 9, 12) for that year

US, ultrasonography; CT, computed tomography; MRI, magnetic resonance imaging; PET, positron emission tomography

Table 2 Results of the questionnaire

Institutions that responded to the questionnaire	17
Doctors who responded to the questionnaire	26
Standardized follow-up program in the institution?	
Yes	6
No	11
Continued follow-up for more than 5 years?	
Yes	15
No	11
Arrange follow-up schedule by disease stage?	
Yes	15
1A/1B,2,3	6
1A/1B,2/3	4
1/2/3	2
1A/1B/2,3	1
1/2,3	1
1,2/3	1
No	11
Physical examination?	
Yes	26
No	0
Blood examination?	
Yes	26
No	0
Sputum cytology?	
Yes	0
No	26
Chest radiography?	
Yes	26
No	0
Abdominal US?	
Yes	0
No	26
CT?	
Yes	26
No	0
Bone scintigraphy?	
Yes	5
No	21
Brain MRI (CT)?	
Yes	10
No	16
PET/CT?	
Yes	7
No	19

the doctors performed physical examination four times a year during the first 3 years and twice a year during the next 2 years. After 5 years of postoperative follow-up, the frequency of the physical examination decreased gradually, although approximately half of the doctors continued the physical examinations once a year for up to 10 years after the resection. The blood examinations were performed almost at the same time points as the physical examination. CT was performed at 6 and 12 months after the resection and was repeated annually thereafter for the next 4 years. Chest radiography was performed at each visit during the first 2 years and repeated annually thereafter, between the CT

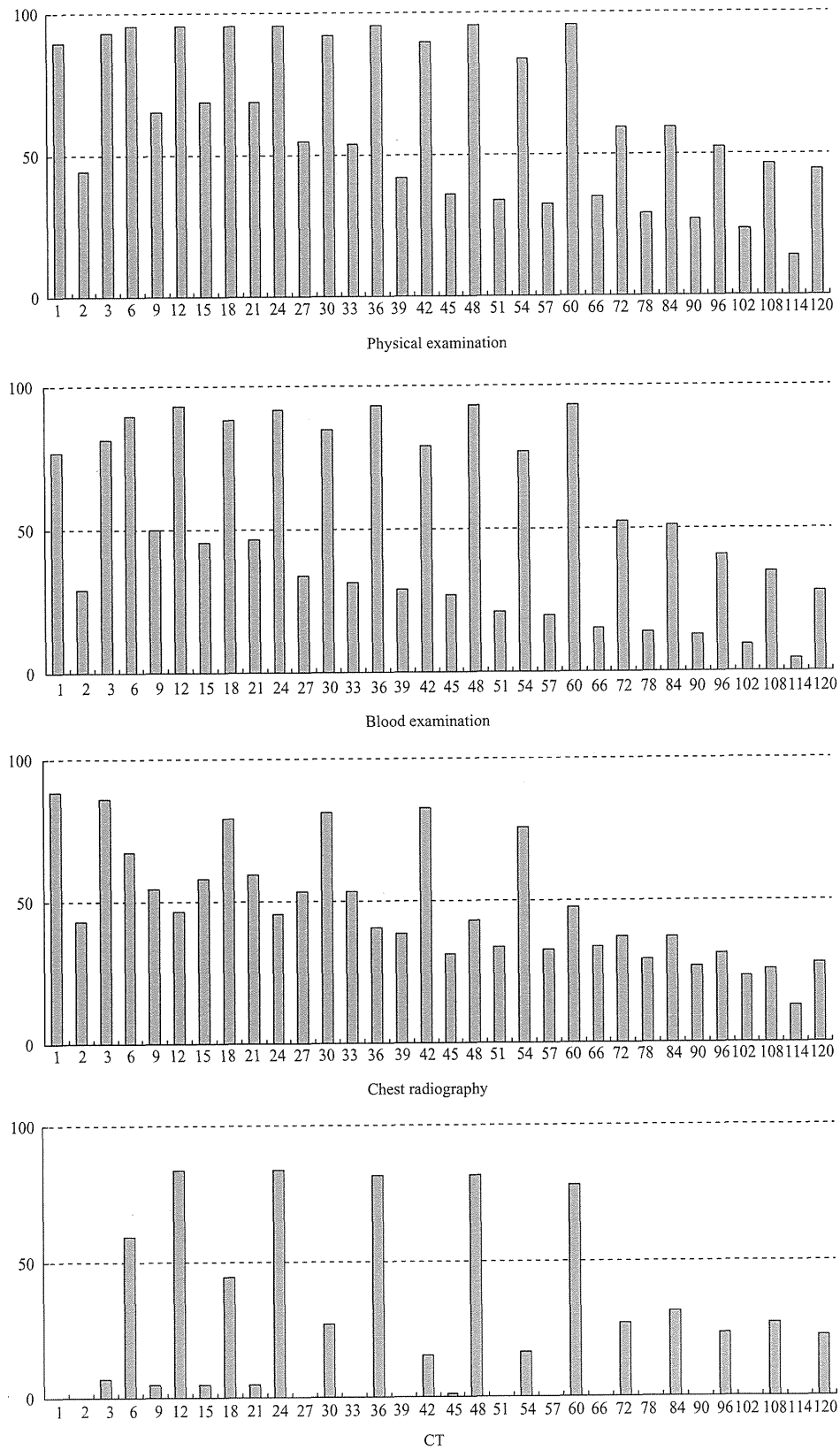
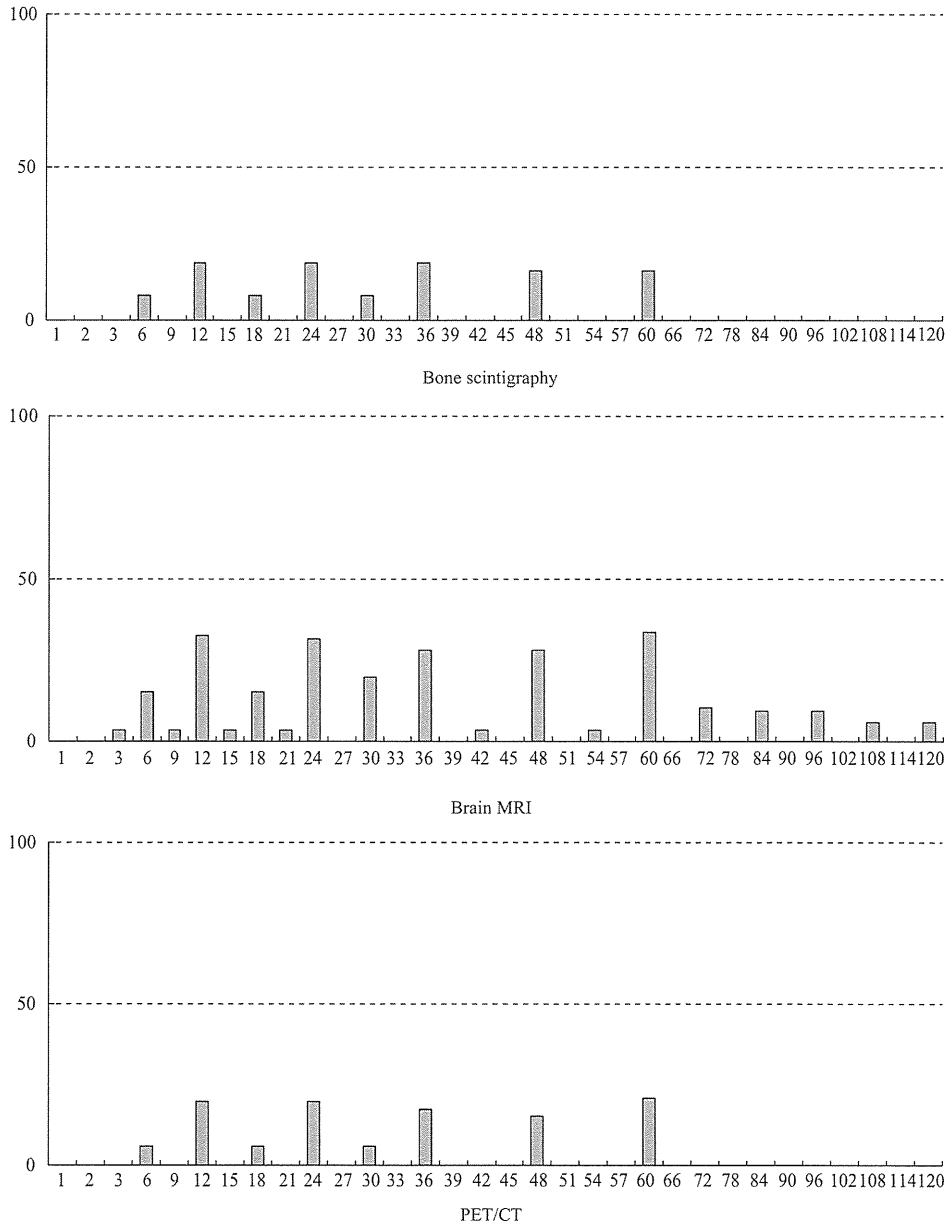


Fig. 1 Frequency of examinations at each time point after resection. *CT*, computed tomography; *MRI*, magnetic resonance imaging; *PET*, positron emission tomography

Fig. 1 Continued



examinations. A few of the doctors routinely performed bone scintigraphy, brain MRI or CT, and PET/CT.

Based on the results of the survey, the examinations that were performed at a frequency of >50% and the time points after resection at which they were performed were selected and are the components of an average follow-up program presented in this study (Table 3): Physical examination was scheduled three to four times a year for the first 3 years, twice a year for the next 2 years, then continued once a year for up to 8 years after the resection. Blood examinations were performed approximately at the same time points as the physical examination. CT examination was scheduled at 6 and 12 months after the resection and repeated annually thereafter for up to 5 years after the resection. Chest radiography was scheduled three or four times a year for the first 3 years and repeated once a year thereafter, between the CT examinations.

Discussion

Several issues need to be discussed in relation to the follow-up of NSCLC patients after resection. One of the

Table 3 Average follow-up program based on the results of this survey

Examination	1 Year						2 Years				3 Years				4 Years				5 Years				6 Years		7 Years		8 Years	
	1	2	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	6	12	6	12	6	12
Physical examination	○		○	○	○	○	○	○	○	○	○	○		○		○		○		○		○	○		○	○		○
Blood examination	○		○	○	○	○		○		○		○	○	○		○	○	○		○		○	○		○	○		
Chest radiography	○		○	○	○		○	○	○		○	○	○			○	○			○								
CT				○		○		○		○				○				○				○						

issues is that optimal examination modalities for the postoperative follow-up have not yet been identified. Several guidelines have been proposed for the follow-up of NSCLC patients after surgery with curative intent, but there is a divergence among the guidelines, especially in relation to the recommendations for imaging examinations such as CT.[11–14] The study presented here demonstrated that follow-up programs applied in clinical practice in Japan also vary widely among institutions and/or doctors in terms of the examinations performed and the frequencies at which they are performed. No optimal or standard examination modalities in the follow-up setting have yet been established.

Another issue pertains to the efficacy and benefits of the follow-up examinations after resection. Several studies have suggested that the survival duration is greater in patients with asymptomatic recurrences than in those with symptomatic recurrences, and that follow-up is useful for detecting asymptomatic recurrences.[2,3] On the other hand, several investigators have reported that the survival benefit of postoperative follow-up is questionable.[4–8] The benefit of follow-up thus remains a controversial subject.

The third issue related to follow-up is cost-effectiveness, which cannot be ignored nowadays when evaluating the efficacy of a certain modality. Several investigators have analyzed the cost-effectiveness of postoperative follow-up and concluded that it is inefficient and that the survival benefit accruing from the follow-up did not justify its cost.[4–6,8] However, these articles were all published from Western countries; and the medical cost for follow-up, social acceptability of the medical cost, and the patients' needs might be different in Japan. Therefore, the cost-effectiveness of the follow-up should be evaluated independently in Japan.

To answer these questions, it would be ideal to conduct a randomized controlled study. However, the lack of standard follow-up modalities makes to it difficult to design a randomized trial. Regarding this point, our survey might give some helpful information for designing such a trial as the survey showed what modalities were commonly used in clinical practice. Another factor that probably makes the randomized trial more difficult to conduct in Japan is ethics. As the first step to evaluate the efficacy of the follow-up, it would be ideal to conduct the randomized trial between a follow-up group and a no follow-up or minimal follow-up group. Follow-up after resection, however, is already commonly performed in clinical practice and no or minimal follow-up would not be acceptable from an ethical point of view—even though there are no recommended follow-up programs and no proven efficacy of follow-up. Considering the present circumstances in Japan, a possible

trial might be comparison between a follow-up group with average modalities and a follow-up group with more intensive modalities, such as the average follow-up modalities + periodical PET/CT.

The follow-up programs identified in this survey seem relatively intensive compared with those that are commonly accepted worldwide. A possible reason might be related to our medical insurance system. All Japanese citizens are covered by public medical insurance, and the cost of the follow-up examinations is not a burden on the patients. This circumstance makes access to hospitals easy and makes the postoperative follow-up examinations relatively intensive. We do not have information about the follow-up programs adopted in other areas of Japan, but we assume that their programs are similar to those presented in this study.

In this survey, one question pertained to "blood examinations (tumor markers)", and it is uncertain what kinds of tumor markers were measured. In the preoperative setting and in cases of advanced/recurrent lung cancer, tumor markers such as carcinoembryonic antigen (CEA) and cytokeratin 19 fragments (Cyfra), among others, are commonly measured as parameters of the tumor aggressiveness or for evaluating the effectiveness of the treatment. In the follow-up setting, therefore, it is assumed that similar tumor markers would be measured.

^{18}F-Fluorodeoxyglucose (FDG)-PET/CT enables examination of the whole body, excluding the brain, in a noninvasive manner; it also can differentiate, if not always definitively, between malignant and benign lesions. Because of these advantages, FDG-PET/CT was applied as one of the follow-up examinations at six of the institutions. Several investigators have reported the usefulness of FDG-PET/CT in the setting of postoperative follow-up for NSCLC.[15–18] However, FDG-PET/CT cannot be recommended commonly in the postoperative follow-up setting because of limitation of availablility in Japan, cost-effectiveness and unknown efficacy.

A total of 15 of the 26 doctors based their follow-up schedules on the disease stage. They performed the examinations less intensively in patients with an early stage of the disease and more intensively in those with more advanced disease. These schedule changes based on the disease stage might be reasonable because recurrence develops more frequently in patients with advanced disease.

Conclusion

A questionnaire designed to obtain information on the follow-up program adopted for NSCLC patients after complete resection was conducted to grasp the current status in our area. The follow-up programs vary widely among institutions and doctors in terms of the examinations performed and the frequencies at which they are performed. The efficacy of the follow-up for NSCLC patients after resection is still unclear, and further studies are needed to answer questions about it.

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ORIGINAL ARTICLE

Prognostic factors for patients in postoperative brain metastases from surgically resected non-small cell lung cancer

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Abstract
Background Postoperative recurrence in non-small cell lung cancer (NSCLC) reduces the life expectancy of patients. In this retrospective study, we investigated the prognostic factors in patients with postoperative brain metastases from surgical resected non-small cell lung cancer (NSCLC).
Methods We conducted a retrospective chart review of patients who had undergone resection for NSCLC between April 2004 and February 2009 and found 65 had experienced postoperative brain metastases by March 2010. We reviewed these patients for clinicopathological information, treatments and responses to treatment, and overall survival.
Results The 5-year survival rate after the diagnosis of brain metastases was 15.4 %. Significantly favorable prognostic factors for patients after a diagnosis of brain metastases included female gender, adenocarcinoma, a small number (1–3) of brain metastases, no extracranial metastasis at the diagnosis of brain metastases, radiation treatment (whole-brain radiation and/or stereotactic irradiation), and local treatment [stereotactic irradiation and/or surgical operation (craniotomy)]. Furthermore, in patients with only brain metastases as the postoperative initial recurrence, the favorable positive prognostic factors included a small number (1–3) of brain metastases, adjuvant chemotherapy, chemotherapy (including adjuvant and other chemotherapy and excluding epidermal growth factor receptor–tyrosine kinase inhibitors), and local treatment.
Conclusions Our study found that the foregoing clinical characteristics in postoperative brain metastases and the administration of treatment contributed to patient life expectancy.

Keywords Prognostic factors · Postoperative brain metastases · Non-small cell lung cancer

Introduction

Approximately 15–30 % of patients with cancer will develop cerebral metastases [1]. One-third of patients with non-small cell lung cancer (NSCLC) will have cerebral metastases [2], and about 50 % of stage IIIA and IIIB NSCLC patients will develop brain metastasis during treatment [3]. Furthermore, in patients with stage II and IIIA NSCLC, even when a complete resection is possible, the 5-year survival rate is still less than 50 % [4, 5] and the median survival time (MST) following relapse after surgery is reported to be 11.5 months [6]. Several studies have investigated the prognostic factors associated with survival after the postoperative recurrence of NSCLC [7, 8].

Systemic chemotherapy, which is commonly accepted as a treatment option for recurrent NSCLC, has improved overall survival and progression-free survival. However, it is difficult to treat brain metastases with these agents because of the blood–brain barrier. The standard treatment for brain metastases has been whole-brain radiotherapy (WBRT), and stereotactic irradiation (STI) has recently emerged as another treatment [9]. Further, surgical resection [10] and epidermal growth factor receptor–tyrosine

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kinase inhibitors (EGFR-TKIs) [11] are also considered to be potential treatment alternatives.

The purpose of this study was to evaluate prognostic factors for patients with brain metastases from surgically resected non-small cell lung cancer.

Patients and methods

Patients

Between April 2004 and February 2009, 967 patients underwent resection for NSCLC and were diagnosed with pathological stage I–III disease in the Department of Thoracic Surgery, Kyoto University Hospital. Of these 967 patients, 65 with postoperative brain metastases were included in this study. The study was approved by the institute's ethics committee. The clinical and pathological data of the patients were obtained from their medical records and imaging tests. Pathological staging was determined by the tumor-node-metastasis (T-N-M) classification system [4]. The pathological diagnosis was made by at least two pathologists from the Department of Diagnostic Pathology, Kyoto University Hospital, according to the Union for International Cancer Control 7th edition of TNM classification.

Follow-up, diagnosis of recurrence, and treatment

Follow-up information until either death or until March 2010 was obtained from the medical records. Follow-up examinations were symptom oriented, but all patients received medical checkups and chest X-rays at least twice per year and also received whole-body computed tomography (CT) including brain CT [some cases received brain magnetic resonance imaging (MRI)] at least once per year. Adjuvant chemotherapy with oral tegafur and uracil (UFT) for stage I disease (generally excluding T1a), and with platinum-based regimens for stage II–III disease, was administered preferentially as a standard, but alternative regimens or observation were selected when the patients refused to receive these standard regimens.

Tumor recurrences were diagnosed by radiologic examinations. Histological confirmation of the diagnosis was performed when feasible. When brain metastases are detected, additional CT of abdomen and bone scan are performed to identify any other metastases. The treatment modalities for recurrent diseases were platinum-based regimens, non-platinum-based regimens (many of these were single-agent chemotherapy), EGFR-TKIs (gefitinib or erlotinib), surgical resection, and radiotherapy for recurrent sites. Among these, the treatment modalities, especially for brain metastases, were radiation therapy (WBRT and STI) and surgical resection (craniotomy). WBRT was generally performed for patients with more than 3 brain metastases, STI was given for 1–3 brain metastases no more than 3.5 cm in diameter, and surgical resection was employed when there were 1–3 resectable brain metastases more than 3.5 cm in diameter. Radiologists and neurosurgeons were consulted when considering the individual performance status and preference to decide on the final treatment.

Statistical analyses

For analysis of overall survival (OS), each patient's survival time began on the date of diagnosis of postoperative brain metastases and ended on the date of death or the last date of follow-up. Each survival rate was calculated using the Kaplan–Meier method.

The impact of the following factors on OS was evaluated: age (less than 65 years vs. 65 or more), gender, smoking index (less than 30 pack-years vs. 30 or more pack-years), histology (adenocarcinoma vs. non-adenocarcinoma), pathological stage (I vs. II–III), number of brain metastases (1–3 vs. 4 or more), time interval until brain metastases occurred after surgery (less than 1 year vs. 1 year or more), extracranial metastasis at the time of diagnosis of brain metastases (yes vs. no), administration of adjuvant chemotherapy (yes vs. no), administration of chemotherapy (including adjuvant and other chemotherapy excluding EGFR-TKIs) (yes vs. no), administration of EGFR-TKIs (yes vs. no), administration of radiotherapy (WBRT and/or STI) (yes vs. no), and local treatment [STI and/or surgical operation (craniotomy)] (yes vs. no). Moreover, in patients with only brain metastases as the initial postoperative recurrence, the diagnostic timing (symptom vs. regular CT or MRI) was also evaluated as a potential prognostic factor.

Univariate survival analyses were performed using the log-rank test and Wilcoxon test because the log-rank test is generally the most appropriate method; however, the Wilcoxon test is more sensitive when the ratio of hazards is higher at early survival times than at later times [12]. The multivariate analyses were performed by a Cox proportional hazards model. P values <0.05 were considered to be statistically significant. All the statistical calculations were executed using the JMP 9 software program (SAS Institute, Cary, NC, USA).

Results

Of the 967 patients, 65 (6.7 %) developed postoperative brain metastases [female:male ratio, 22/43; age range, 35 to 88 years old (mean, 65.5 years; median, 64 years). Of these 65 patients, 31 had only brain metastases as the initial

postoperative recurrence [female:male ratio, 9/22; age range, 44 to 83 years old (mean age, 61.5 years; median, 62 years).

Patients with postoperative brain metastases

The median overall survival (OS) of the cases with brain metastases ($n = 65$) was 16.3 months and the 5-year survival was 15.4 %. The period between surgery for the primary tumor and brain metastases was from 1.9 to 100.8 months with a mean of 20.9 months and a median of 17.8 months. The mean follow-up period was 20.9 months. Patient characteristics are summarized in Table 1. Regarding the type of surgery for the primary tumor, 5 patients underwent pneumonectomy, 53 lobectomy, and 7 segmentectomy with regional lymph node dissection. As pathological stage at the time of resection for NSCLC, the largest number of patients had stage IIIa disease ($n = 26$). In all 65 patients, the univariate analysis showed that female gender (log-rank, Wilcoxon test: $P = 0.1902$, 0.0311, respectively), adenocarcinoma ($P = 0.0234$, 0.0295), a small number (1–3) of brain metastases ($P = 0.0290$, 0.1344), no extracranial metastasis at the diagnosis of brain metastases ($P = 0.0088$, 0.0066), radiation treatment (WBRT and/or STI) ($P = 0.0145$, 0.0036) (Fig. 1a), and local treatment [STI and/or surgical resection (craniotomy)] ($P = 0.0006$, 0.0003) (Fig. 1b) were significant factors associated with a better prognosis for survival after a diagnosis of brain metastases (Table 2). A multivariate analysis showed that adenocarcinoma ($P = 0.033$, hazard ratio: 2.428), a large number (≥ 4) of brain metastases ($P = 0.039$, hazard ratio: 2.194) extracranial metastasis ($P = 0.023$, hazard ratio: 2.433), and the absence of local treatment ($P = 0.009$, hazard ratio: 2.530) were significant factors associated with a poor prognosis (Table 3).

Patients with only brain metastases as the initial postoperative recurrence

We studied 31 cases who had only brain metastases as the initial postoperative recurrence for subgroup analysis. The distribution of the patient characteristics is summarized in Table 3. In these patients, univariate analysis showed that a small number (1–3) of brain metastases (log-rank, Wilcoxon test: $P = 0.1398$, 0.0236, respectively), adjuvant chemotherapy ($P = 0.0118$, 0.0118), chemotherapy ($P = 0.0002$, 0.0007), and local treatment ($P = 0.0562$, 0.0163) were significant predictors of a better prognosis for postoperative survival after a diagnosis of brain metastases (Table 4). A multivariate analysis showed that only the absence of chemotherapy ($P = 0.027$, hazard ratio: 10.151) was significantly associated with a poor prognosis (Table 5).

Table 1 Patient characteristics ($n = 65$)

Characteristic	Category	Number of patients
Age (years)	<65	27
	≥65	38
Gender	Male	43
	Female	22
Smoking index (pack-year)	<30	27
	≥30	29
	Unknown	9
Operation	Lobectomy	53
	Segmentectomy	7
	Pneumonectomy	5
Histology	Adenocarcinoma	46
	Other	19
Pathological stage	Ia	9
	Ib	9
	IIa	15
	IIb	5
	IIIa	26
	IIIb	1
Number of brain metastases	1–3	41
	≥4	20
	Unknown	4
Interval to brain metastases after surgery		
	<1 year	23
	≥1 year	42
Extracranial metastasis[a]	Yes	34
	No	31
Adjuvant chemotherapy	Yes	56
	No	7
	Unknown	2
Chemotherapy[b]	Yes	53
	No	12
EGFR-TKI[c]	Yes	25
	No	40
Radiotherapy[d]	Yes	54
	No	11
Local treatment[e]	Yes	31
	No	34

[a] At the time of the diagnosis of brain metastases

[b] Including adjuvant and other types of chemotherapies and excluding EGFR-TKI

[c] Epidermal growth factor receptor–tyrosine kinase inhibitor (EFGR-TKI)

[d] Whole-brain radiation and/or stereotactic irradiation

[e] Stereotactic irradiation and/or surgical operation (craniotomy)

Discussion

In this study of patients with postoperative brain metastases, the significant positive prognostic factors for

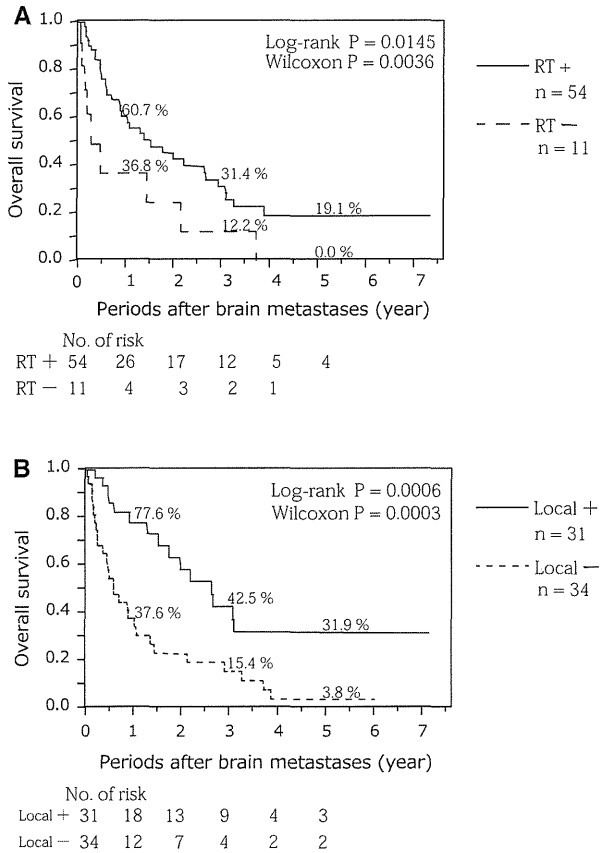

Fig. 1 Kaplan–Meier curve depicting the effect of treatments on survival time begun on the date of diagnosis of postoperative brain metastases. Percentages on the curve show 1-, 3-, and 5-year survival rates. *RT* radiation therapy (whole-brain radiation and/or stereotactic irradiation), *Local* local therapy [stereotactic irradiation and/or surgical operation (craniotomy)]

Table 2 Results of univariate analyses of the prognostic factors for overall survival after brain metastases in patients with postoperative brain metastases

Variable	High-risk group	*P* value (log-rank/Wilcoxon)
Age (years)	≥65	0.1614/0.2533
Gender	Male	0.1902/0.0311
Smoking index (pack-years)	≥30	0.2626/0.1174
Histology	Non-adenocarcinoma	0.0234/0.0295
Pathological stage	II–III	0.7659/0.7605
Number of brain metastases	≥4	0.0290/0.1344
Time interval until brain metastases after surgery	<1 year	0.8518/0.6889
Extracranial metastases[a]	Yes	0.0088/0.0066
Adjuvant chemotherapy	No	0.1837/0.3404
Chemotherapy[b]	No	0.5853/0.2082
EGFR-TKI[c]	No	0.4531/0.2951
Radiation therapy[d]	No	0.0145/0.0036
Local treatment[e]	No	0.0006/0.0003

[a] At the time of the diagnosis of brain metastases

[b] Including adjuvant and other types of chemotherapies and excluding EGFR-TKI

[c] Epidermal growth factor receptor–tyrosine kinase inhibitor

[d] Whole-brain radiation and/or stereotactic irradiation

[e] Stereotactic irradiation and/or surgical operation (craniotomy)

postoperative survival after a diagnosis of brain metastases included female gender, adenocarcinoma histology, a small number (1–3) of brain metastases, and no extracranial metastasis at the diagnosis of the brain metastases, radiation treatment (WBRT and/or STI), and local treatment [STI and/or surgical resection (craniotomy)]. Furthermore, in patients with only brain metastases as the initial postoperative recurrence, the significant positive prognostic factors included a small number (1–3) of brain metastases, presence of adjuvant chemotherapy, presence of chemotherapy (including adjuvant and other chemotherapy and excluding epidermal growth factor receptor–tyrosine kinase inhibitors), and local treatment.

Gender (female) and histology (adenocarcinoma) were among the factors associated with a better prognosis for patients with postoperative brain metastases both with and without extracranial metastases in our study, which agrees with the trends of overall [13] and resected [14] NSCLC. Furthermore, radiation therapy (WBRT and/or STI) was also a favorable prognostic factor; this was in agreement with other studies of subjects with inoperable primary lung cancer or uncontrollable extracranial metastases, which revealed the efficacy of radiation therapy, including WBRT [15, 16] and STI [15–23].

We found that lack of extracranial metastasis at the initial discovery of the brain metastases was a significantly favorable finding, which seems to be in accord with other reports which showed that control in the extracranial region improved the overall survival [23, 24]. Therefore, these findings suggest that control of the extracranial region is also very important in cases with brain metastases after pulmonary resection. Furthermore, our multivariate analysis in patients with only brain metastases as the initial postoperative recurrence (no extracranial metastases) suggest that they should be exhaustively treated with local treatments to prolong their life expectancy.

In the patients with only brain metastases as the initial postoperative recurrence, our multivariate study revealed that a small number (1–3) of brain metastases and local treatment were favorable prognostic factors. Recent studies have shown that a small number of brain metastases could be treated by STI and surgical resection. Several retrospective trials of STI and one retrospective study on surgical resection [10] included patients who benefited from

Table 3 Results of multivariate analyses of the prognostic factors for overall survival after brain metastases in patients with postoperative brain metastases

Variable	High-risk group	*P* value	Hazard ratio	95 % confidence interval
Gender	Male	0.250	0.614	0.254–1.409
Histology	Non-adenocarcinoma	0.033	2.428	1.077–5.222
Number of brain metastases	≥4	0.039	2.194	1.041–4.572
Extracranial metastases[a]	Yes	0.023	2.433	1.129–5.384
Radiation therapy[b]	No	0.638	1.315	0.400–3.985
Local treatment[c]	No	0.009	2.530	1.260–5.211

[a] At the time of the diagnosis of brain metastases

[b] Whole-brain radiation and/or stereotactic irradiation

[c] Stereotactic irradiation and/or surgical operation (craniotomy)

Table 4 Results of univariate analyses of the prognostic factors for overall survival in patients ($n = 31$) with only brain metastases as the initial postoperative recurrence

Variable	Categories[a]	*P* value (log-rank/Wilcoxon)
Age	≥65 (14)/<65 (17)	0.0945/0.0708
Gender	Male (22)/female (9)	0.2456/0.0997
Smoking index[b] (pack-years)	≥30 (16)/<30 (12)	0.4157/0.3344
Histology	Non-adenocarcinoma[e] (10)/adenocarcinoma (21)	0.1489/0.1271
Number of brain metastases[b]	≥4 (11)/1–3 (19)	0.1398/0.0236
Time interval until brain metastases after surgery	<1 year (14)/≥ 1 year (17)	0.2291/0.2588
Diagnostic timing	Symptoms (22)/regular CT or MRI (9)	0.7719/0.4679
Adjuvant chemotherapy[b]	No (5)/yes (25)	0.0118/0.0118
Chemotherapy[c]	No (7)/yes (24)	0.0002/0.0007
EGFR-TKI[d]	No (23)/yes (8)	0.1327/0.1361
Radiation therapy[e]	No (1)/yes (30)	0.8501/0.8501
Local treatment[f]	No (12)/yes (19)	0.0562/0.0163

[a] Described as the "high-risk group (number)/low risk group (number)"

[b] Number includes unknown cases

[c] Including adjuvant and other types of chemotherapies and excluding EGFR-TKI

[d] Epidermal growth factor receptor–tyrosine kinase inhibitor

[e] Whole-brain radiation and/or stereotactic irradiation

[f] Stereotactic irradiation and/or surgical operation (craniotomy)

Table 5 Results of multivariate analyses of the prognostic factors for overall survival in patients ($n = 31$) with only brain metastases as the initial postoperative recurrence

Variable	High-risk group	*P* value	Hazard ratio	95 % confidence interval
Number of brain metastases	≥4	0.460	1.607	0.434–5.509
Adjuvant chemotherapy	No	0.249	2.883	0.450–15.869
Chemotherapy[a]	No	0.027	10.151	1.304–100.423
Local treatment[b]	No	0.429	1.640	0.465–5.630

[a] Including adjuvant and other types of chemotherapies and excluding EGFR-TKI

[b] Stereotactic irradiation and/or surgical operation (craniotomy)

aggressive local treatment [17, 19, 22, 23, 25]. Furthermore, new lesions can be treated with repeated gamma knife surgery as local treatment [24]. In contrast, the conventional standard treatment for brain metastases has been WBRT [26], and a prophylactic effect was reported in NSCLC, as in small cell lung cancer, for this modality.