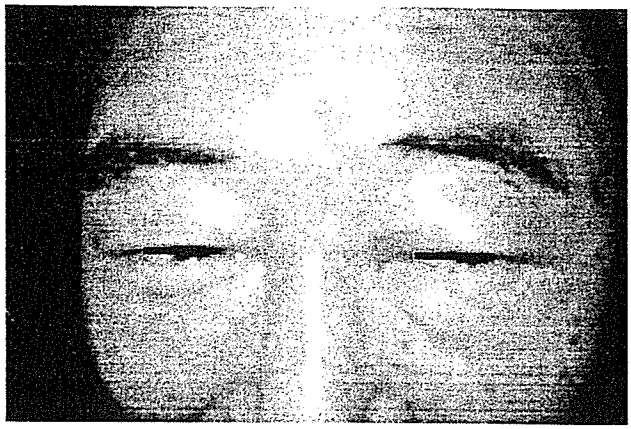

(A)

(B)

Fig. 1 A 56-year-old man with adenocarcinoma of the lung. Edema of the eyelid appeared on day 8 of the second course of pemetrexed. (A) Photograph taken from the front. (B) Profile.

dose: 3900 mg/body) (Fig. 1). He developed no other type of edema. He had no hypoproteinemia or did not undergo hydration. Initially, cardiac failure and conjunctivitis were considered possible causes. A diuretic was given, but did not improve the edema. The edema was therefore thought to be a side effect of pemetrexed, and 8 mg dexamethasone was administered. The edema was dramatically improved 6 days after administration of steroid (Fig. 2). Since the tumor had decreased in size, administration of pemetrexed was continued. The eyelid edema appeared whenever a course of pemetrexed was repeated. This edema was therefore considered probably related to pemetrexed.

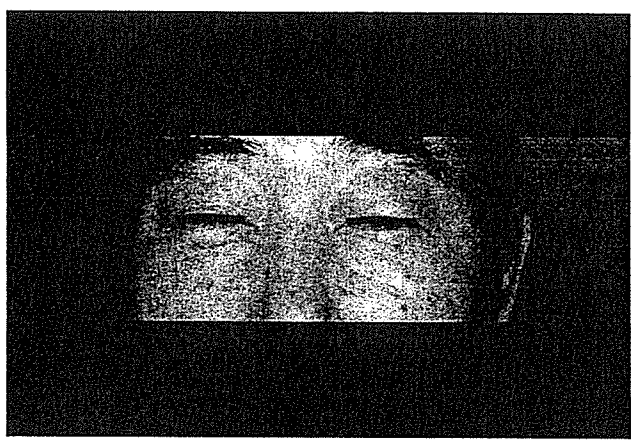

Fig. 2 The edema of the eyelid was improved by the administration of corticosteroid.

3. Discussion

Pemetrexed-associated edema of the eyelid has been previously reported in only one case (0.2%, $n = 519$), according to the Pemetrexed Clinical Investigator's Brochure, April 2005 version. The mechanism responsible for this severe swelling is unknown. Similarly, docetaxel has also been documented to cause peripheral edema. Recently, Semb et al. [5] reported that docetaxel enhances fluid filtration, followed by capillary protein leakage that causes edema and nonmalignant effusion. Prophylactic administration of corticosteroid during docetaxel administration appears to delay and decrease the severity of these adverse events. It may be that pemetrexed-induced eyelid edema is due to the same mechanism as the edema produced by docetaxel.

There are still unanswered questions regarding this drug-induced eyelid edema. Why is it confined to the eyelid? Is it a cumulative adverse event? We believe that medical oncologists should be aware of this rare adverse event and attempt to determine its cause.

Conflict of interest statement

None declared.

Acknowledgment

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ORIGINAL ARTICLE

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Non-small cell lung cancer: radiation therapy for locoregional recurrence after complete resection

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Abstract

Background. We investigated patterns of failure after radical radiation therapy in relation to the radiation field in patients with postsurgical locoregional recurrence of non-small cell lung cancer.

Methods. Between 1992 and 2002, 31 patients with locoregional recurrence were treated with radiation therapy. At the time of radiation therapy, the sites of recurrence were the bronchial stump, the regional lymph nodes, the chest wall, and both the regional lymph nodes and the chest wall in 7, 20, 3, and 1 patient, respectively. The prescribed dose was 60 Gy in 30 fractions over 6 weeks in all patients.

Results. The response rate was 87%. The overall 1-year, 2-year, and 4-year Kaplan-Meier survival rates were 61%, 30%, and 15%, respectively, and the median survival time was 14 months. Locoregional relapse with or without distant metastasis occurred in 15 patients (in-field, 7; marginal, 7; out-field, 1), and distant metastasis alone occurred in 7 patients. The sites of marginal relapse were the upper margin in two patients, the ipsilateral margin in one patient, the contralateral margin in one patient, and the lower margin in three patients, respectively (in one patient, the data for marginal relapse overlapped). In all patients with relapse on the lower margin, the mediastinal lymph nodes were dissected at the initial surgery.

Conclusion. Postoperative recurrent non-small cell lung cancer showed distinctive features: the response rate was high, and the incidence of marginal relapse was also high, as in small cell lung cancer. The incidence of lower marginal relapse was high, in contrast to that in surgery-naive patients.

Key words Non-small-cell lung cancer · Radiation therapy · Surgery · Recurrence

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Introductions

Stereotactic radiotherapy is rapidly spreading as a definitive treatment for stage I non-small cell lung cancer.[1] However, until recently, surgery has been a standard treatment for patients with early stage non-small cell lung cancer. After surgery, 5%–20% of patients develop locoregional recurrence as the first site of the failure.[2–5] For locoregional recurrence, radiation therapy is the treatment of choice, and several reports have shown that 2- and 5-year survival is comparable to those for radiation therapy alone in patients with primary stage III non-small cell lung cancer.[6–8] Therefore, we have treated these patients with radical radiation therapy when possible.

To investigate the role of radical radiation therapy in this patient population, the data were reviewed for a single institution. In particular, patterns of failure in relation to the radiation field were investigated.

Patients and methods

Eligible for the current analysis were patients with locoregional recurrence of non-small cell lung cancer after curative surgery. Patients with distant metastasis or contralateral hilar lymph node metastasis were excluded from this analysis. Between 1992 and 2002, 31 eligible patients were treated with radical radiation therapy in our

Table 1. Characteristics of patients

Characteristics	Number of patients
Sex	
Male	26
Female	5
Age (median, 68 years; range, 44–83 years)	
<70 years	16
≥70 years	15
Histology	
Squamous cell carcinoma	20
Adenocarcinoma	9
Other	2
ECOG performance status	
0–1	26
2	4
3	1
Surgery	
Lobectomy	24
Pneumonectomy	6
Wedge resection	1
Recurrence site	
Stump	7
Regional lymph node	
N2	13
N3	8
Peripheral	4
Longest diameter of recurrent tumor	
8–19 mm	3
20–39 mm	14
40–59 mm	12
60–85 mm	2

ECOG, Eastern Cooperative Oncology Group
Recurrence sites overlap in one patient

institution. Oral informed consent was obtained from all patients.

Initial surgery was lobectomy in 24 patients (78%), pneumonectomy in 6 patients (19%), and wedge resection in 1 patient (3%). The mediastinal lymph nodes were dissected in 23 patients (74%). The median interval between initial surgery and radiation therapy was 15 months (range, 4–61 months).

Characteristics of the patients at the time of radiation therapy are summarized in Table 1. Recurrence was histologically diagnosed in 20 patients (65%). In other patients, obvious enlargement of the tumor was confirmed by postoperative follow-up computed tomography (CT). The sites of recurrence were the bronchial stump, the regional lymph nodes, the chest wall, or both the regional lymph nodes and the chest wall in 7 (23%), 20 (64%; N2, 12; N3, 8), 3 (10%), and 1 (3%; N2) patient, respectively. The longest diameter of the recurrent tumor, measured on CT, is also presented in Table 1.

Irradiation was performed with 10 MV photons from a linear accelerator. Lung density correction was not performed. The prescribed dose was 60 Gy in 30 fractions over 6 weeks in all patients. The radiation field contained the ipsilateral hilar lymph nodes and the mediastinal lymph nodes (from the subcarinal lymph nodes to the upper mediastinal lymph nodes) in 26 (84%) and 18 (58%) patients, respectively. Elective mediastinal irradiation was often omitted in patients with supraclavicular lymph node metastasis alone, or in patients who had undergone pneumonectomy. In these patients, the radiation field contained the recurrent tumor and margins of more than 20 mm. When the initial radiation field contained the spinal cord, off-cord (i.e., the spinal cord was outside the field) oblique boost fields were used after initial irradiation with a dose of 30 Gy or 40 Gy. Chemotherapy was performed sequentially or concurrently in five patients. The agents are listed in Table 2.

Table 2. Agents in chemotherapy

Characteristics	Number of patients
Cisplatin + vindesine	1
Gemcitabine + paclitaxel	1
Carboplatin + paclitaxel	1
Cisplatin + vinorelbine	1
Docetaxel	1

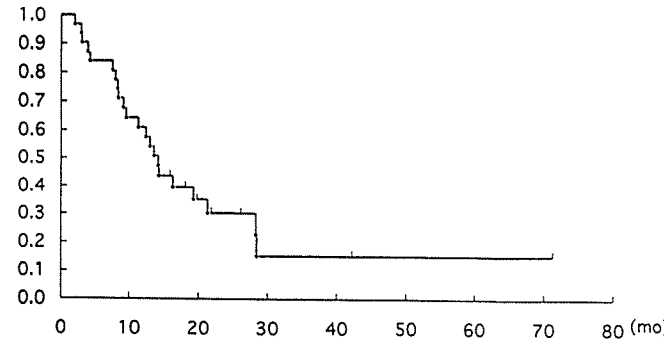

Fig. 1. Kaplan-Meier survival curve

Survival was calculated using the Kaplan-Meier method, and the differences between the curves were analyzed using the generalized Wilcoxon method. Tumor response to irradiation was evaluated with CT. A complete response (CR) was defined as 100% regression of the tumor, and a partial response (PR) was defined as more than 50% regression of the tumor, when evaluated 0–6 months after irradiation.

Results

One patient could not receive the full dose of radiation therapy owing to the presence of a bronco-esophageal fistula. The overall 1-year, 2-year, and 4-year Kaplan-Meier survival rates were 61%, 30%, and 15%, respectively, and the median survival time was 14 months (Fig. 1). The response rate was 87% (CR, 23%; PR, 64%).

The median survival times according to various prognostic factors are summarized in Table 3. The median survival time among patients with recurrence in the bronchial stump and the regional lymph nodes was 16 months and 13 months, respectively (generalized Wilcoxon, $P = 0.28$). No correlations were found between survival and extent of initial surgery, tumor histology, or radiation field.

Locoregional relapse with or without distant metastasis occurred in 15 patients, and distant metastasis alone occurred in 7 patients. Local relapse was subgrouped accord-

Table 3. Median survival time (MST) according to various prognostic factors

Factor	MST (months)	P
Age		
<70	16	0.06
≥70	9	
Histology		
Squamous cell carcinoma	14	0.66
Adenocarcinoma	14	
Performance status		
0–1	16	0.01
2–3	4	
Surgery		
Lobectomy	14	0.85
Pneumonectomy	14	
Recurrence site		
Stump	16	0.11
N2	9	
N3	19	
Radiation field (mediastinum)		
Yes	12	0.07
No	21	

P value for the recurrence site was between the stump and N2 lymph node metastasis

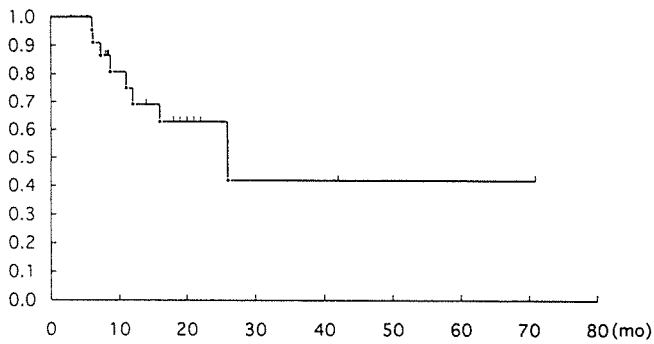

Fig. 2. Kaplan-Meier curve of the in-field control

ing to in-field relapse, marginal relapse, or out-field relapse, that is, relapse with respect to the radiation field (marginal relapse was defined as locoregional relapse at the edge of the radiation field). In-field relapse, marginal relapse, and out-field relapse occurred in seven, seven, and one patient, respectively (the out-field relapse was ipsilateral hilar lymph node metastasis; the lymph nodes had not been contained in the radiation field). The sites of marginal relapse were the upper margin in two patients, the ipsilateral margin in one patient, the contralateral margin in one patient, and the lower margin in three patients, respectively (in one patient, the data for marginal relapse overlapped). In four of the seven patients with marginal relapse, the radiation field contained the mediastinal lymph nodes. In all patients with relapse on the lower margin, the mediastinal lymph nodes were dissected at the initial surgery. The 2-year and 4-year in-field control rates were 62% and 41%, respectively (Fig. 2).

Discussion

There are several reports on the role of radiation therapy in the treatment of patients with postoperative locoregional recurrent non-small cell lung cancer. Although the number of patients was not large in the current study, the prescribed dose was uniform and patterns of recurrence in relation to the radiation field were investigated.

In surgery-naive patients, marginal relapse after radiation therapy occurred in 4% and 16% of patients with non-small cell lung cancer and with small cell lung cancer, respectively, in our institution.[9,10] However, the presented results showed that marginal relapse occurred in 23% of the patients with postoperative locoregional recurrent non-small cell lung cancer. A narrow radiation field did not cause the frequent marginal relapse since among 18 patients with the conventional radiation field, which contained the mediastinal lymph nodes, marginal relapse occurred in 22%. Furthermore, the response rate was 87%, which is higher than the usual response rate in surgery-naive non-small cell lung cancer. These features were similar rather to those of small cell lung cancer. However, causes for the distinctive features are unclear; invasively spread tumors might be specific to this population, or the nature of the tumor may have been changed by surgery.

In patients with surgery-naive small cell lung cancer, marginal relapse frequently occurs on the upper margin of the radiation field.[10] However, in the current study, the incidence of lower marginal relapse was high. In all patients with lower marginal relapse, the mediastinal lymph nodes were dissected. Therefore, a change in lymphatic circulation by surgery is considered to have caused the lower marginal relapse.

The median survival time of 14 months and the 2-year survival of 30% are comparable to results for radiation therapy alone in patients with surgery-naive locally advanced non-small cell lung cancer.[11,12] Therefore, radiation therapy is considered to play a role in the treatment of postoperative recurrent non-small cell lung cancer. However, the role of radiation therapy will be changed by progress in surgical techniques or in imaging techniques used for diagnosis, such as positron emission tomography.[13,14]

In conclusion, postoperative recurrent non-small cell lung cancer showed distinctive features: the response rate was high, and the incidence of marginal relapse was also high, similar to those of small cell lung cancer. The incidence of lower marginal relapse was high, in contrast to that in surgery-naive patients.

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Hepatoma-derived growth factor as a prognostic marker in completely resected non-small-cell lung cancer

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Abstract. Hepatoma-derived growth factor (HDGF), unrelated to hepatocyte growth factor, is a heparin-binding protein originally purified from human hepatoma HuH-7 cells. HDGF exhibits mitogenic activities for certain hepatoma cells, fibroblasts and vascular smooth muscle cells, and angiogenic activities through nuclear targeting. Recently, HDGF was found to be a mitogen for lung epithelial cells *in vitro* and *in vivo*. This suggests that HDGF may play a critical role in the development and progression of lung cancer. We investigated, immunohistochemically, the relationship between HDGF expression and clinicopathological variables, and the prognostic significance of HDGF in 102 patients with completely resected non-small-cell lung cancer (NSCLC: 70 adenocarcinomas and 32 squamous cell carcinomas). To address the mechanism of action of HDGF, we evaluated the contribution of HDGF to tumor cell proliferation and intratumor angiogenesis using anti-Ki-67 and anti-CD31 antibodies, respectively. HDGF expression was strongly detected in the nucleus of cancer cells; the HDGF-labeling index (LI) was 20-95% (median 64.5%). There was no significant association between HDGF-expression level and clinicopathological variables. Patients with NSCLC showing a high HDGF-LI (≥65%) had significantly worse overall and disease-free survivals than those with NSCLC showing a low HDGF-LI. Multivariate analysis revealed that HDGF is a significant independent prognostic factor, more powerful than pathological stage. Moreover, HDGF expression correlated with Ki-67-LI and intratumor microvessel density. We consider HDGF as a useful prognostic marker for patients with completely resected NSCLC and it may play a critical role in the pathobiology of lung cancer through its mitogenic and angiogenic activities.

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Key words: hepatoma-derived growth factor (HDGF), non-small-cell lung cancer, prognostic factor, immunohistochemical study, microvessel density

Introduction

Hepatoma-derived growth factor (HDGF), unrelated to hepatocyte growth factor (HGF) produced by non-parenchymal cells, is a secretory heparin-binding protein that was purified from the conditioned medium of human hepatoma HuH-7 cells, and its cDNA was cloned from HuH-7 cells (1,2). HDGF represents a new family of growth factors called HDGF-related proteins (HRPs), including HRP1, HRP2, HRP3, HRP4 and p52/p75/lens epithelium-derived growth factor (LEDGF) (3). These proteins have in common the following characteristics: i) homology in the N-terminal amino acids [termed homologous to the amino terminus of HDGF (hath) region] containing a PWWP domain, which is suspected to play a role in cell growth and differentiation possibly by DNA binding, ii) bipartite nuclear localization signals, and iii) lack of signal peptides (3-5). Recent studies have shown that HDGF is an exogenous mitogen for HuH-7, Swiss 3T3 fibroblasts (2), endothelial cells (6-8), and vascular smooth muscle cells (9,10), and that nuclear targeting of HDGF is essential for its mitogenic activity (10,11).

As for roles of HDGF in tumor pathobiology, HDGF stimulates *in vitro* proliferation of hepatoma cells such as HuH-7, and antisense oligonucleotides of HDGF can suppress it (12). *In vivo*, HDGF induces tumorigenesis of NIH3T3 cells in nude mice through its angiogenic activity (7) and may also play an important role in the development and progression of hepatocellular carcinoma in humans and rodents on the basis that HDGF expression is higher in hepatoma cells than in the adjacent non-cancerous tissues (13).

Although HDGF was originally identified in hepatoma cells, HDGF and its mRNA are expressed in various normal adult tissues, including lung tissue (2,6,14). HDGF may be involved in fetal lung development (15). Recently, Mori *et al* (14) reported that HDGF is also a mitogen for lung epithelial cells *in vitro* and *in vivo*. Taken together, these findings suggest that HDGF may play a critical role in the development and progression of lung cancer.

The most common cancer in Japan today is lung cancer. Lung cancer was the leading indication for general thoracic

surgery (~43%) and more than 20,000 patients were operated on at Japanese institutions in 2002 (16). Non-small-cell lung carcinomas (NSCLC) represent 98% of all operable cases of lung cancer, and they are still associated with a poor prognosis, even when operable. Many molecular markers of prognosis have been studied, although the critical cause for the poor prognosis of patients with NSCLC remains to be determined.

In the present study, we investigate immunohistochemically the relationship between HDGF expression and clinicopathological variables and the prognostic significance of HDGF in NSCLC patients who underwent complete resection. Additionally, to address the mechanism of action of HDGF on lung cancer biology, we evaluated the contribution of HDGF to tumor cell proliferation and intratumor angiogenesis.

Materials and methods

Patients and tumors. Among patients with primary lung carcinoma who were operated on at the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases (Osaka, Japan) from 1994 through 1997, one hundred and two patients underwent complete resection for adenocarcinoma (n=70) or squamous cell carcinoma (n=32) without previous chemotherapy or radiotherapy, and adequate paraffin-embedded tissue sections were available. These patients had no other form of malignancy. Tumor specimens were fixed in 10% formaldehyde solution, embedded in paraffin and microscopically examined after hematoxylin and eosin (HE) staining. Histological classification of tumors was based on the World Health Organization criteria. Visceral pleural involvement was classified according to the Japan Lung Cancer Society (17) as follows: P0, the tumor does not penetrate the elastic layer of the visceral pleura; P1, the tumor penetrates the elastic layer but is not exposed on the pleural surface; P2, the tumor is exposed on the pleural surface but does not involve adjacent anatomic structures; and P3, the tumor involves adjacent anatomic structures (18). A tumor larger than 3 cm in diameter or a P2 tumor of any size was defined as T2 classification. All tumors were staged according to the TNM pathological classification of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) (19): 40 stage I (23 cases in stage IA and 17 cases in stage IB), 21 stage II (3 cases in stage IIA and 18 cases in stage IIB), 35 stage III (26 cases in stage IIIA and 9 cases in stage IIIB) and 6 stage IV (patients with a metastatic nodule in the ipsilateral non-primary-tumor lobe of the lung). The patients (69 men and 33 women) were between 40 and 80 years of age (mean 64 years) and grouped according to age as being either <70 or ≥70 years old. Smoking status was 0-232 (median 44.5) pack-year, and patients were divided into 2 groups: those who smoked <40 pack-year and those who smoked ≥40 pack-year. Survival was calculated from the day of surgery, and follow-up of the 102 patients ranged from 4.1 to 108.9 (median 61.3) months; 54 patients (52.9%), without exception, died of recurrence or metastasis of lung cancer during follow-up. Our study was carried out with the approval of the ethical committee of the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases.

Immunohistochemical examination. Immunohistochemical staining for HDGF was performed essentially as previously described (7,12,14,20). The paraffin sections (4 μm thick) were deparaffinized, microwaved in 10 mmol/l citrate buffer (pH 6.0) and then immersed in methanol containing 0.3% hydrogen peroxide. Slides were blocked with normal goat serum and incubated with a 1:5,000 dilution of rabbit polyclonal IgG raised against C-terminus (231-240) of the human HDGF sequence for 30 min at room temperature. After washing the sections twice with phosphate-buffered saline, they were incubated with peroxidase-conjugated goat anti-rabbit immunoglobulin (Envision; Dako, Glostrup, Denmark) for 30 min at room temperature. After washing, diaminobenzidine tetrahydrochloride (DAB) solution was applied. The sections were then counterstained in hematoxylin. Specificity of the anti-HDGF antibody (Ab) had been previously demonstrated by Western blot analysis using recombinant human HDGF (14). Weak staining of smooth muscle cells and endothelial cells of blood vessels was used as the internal positive control. Negative controls were treated in the same way, but anti-HDGF Ab was replaced by non-immune rabbit serum. HDGF was detected mainly in the nucleus of cancer cells more strongly than in that of smooth muscle cells, and weakly in the cytoplasm of some cancer cells. HDGF immunoreactivity was judged positive when HDGF staining in the nucleus of tumor cells was equivalent to or stronger than that in the nucleus of smooth muscle cells. HDGF-labeling index (LI) was expressed as the proportion of cancer cells with positive HDGF nuclear reactivity.

Immunohistochemical staining for Ki-67 nuclear antigen was performed using a mouse monoclonal anti-human Ki-67 antigen Ab (MIB-1, DAKO) according to the manufacturer's instructions. Ki-67-LI was expressed as the proportion of Ki-67-positive cancer cells. For evaluation of HDGF- and Ki-67-LI, more than 1,000 cancer cells were counted in at least 5 representative areas without necrosis in each section. Intratumor angiogenesis was assessed by counting the microvessels detected with CD31 staining using a mouse monoclonal anti-human CD31 Ab (JC/70A, DAKO) according to the manufacturer's instructions. Intratumoral microvessel density (MVD) was calculated as the average value of microvessels/mm^2 using the criteria previously described elsewhere (21,22). After the area of highest vascularization was identified by scanning sections at low power, individual microvessel counts were determined at magnification x200 (0.95 mm^2 area) in 3 different fields under an Olympus microscope (Tokyo, Japan). All values determined by slide examination were presented by the median of scores evaluated by 3 investigators (Teruo Iwasaki, Yoshiaki Takada and Kunimitsu Kawahara).

Statistical analysis. The relationship between HDGF expression and clinicopathological variables [age, sex, smoking, tumor size, pathological stage, T-factor (classification), N-factor (classification), pleural involvement, vascular involvement, lymphatic involvement, histological type and degree of differentiation] was analyzed by the χ^2-test. The significance of differences in Ki-67-LI and MVD was tested by Student's t-test. The Kaplan-Meier method was used to estimate overall and disease-free survival as a function of time, and survival differences were analyzed by the log-rank test. Factors potentially related to overall and disease-free survival were analyzed by the Cox proportional-hazards model. For all

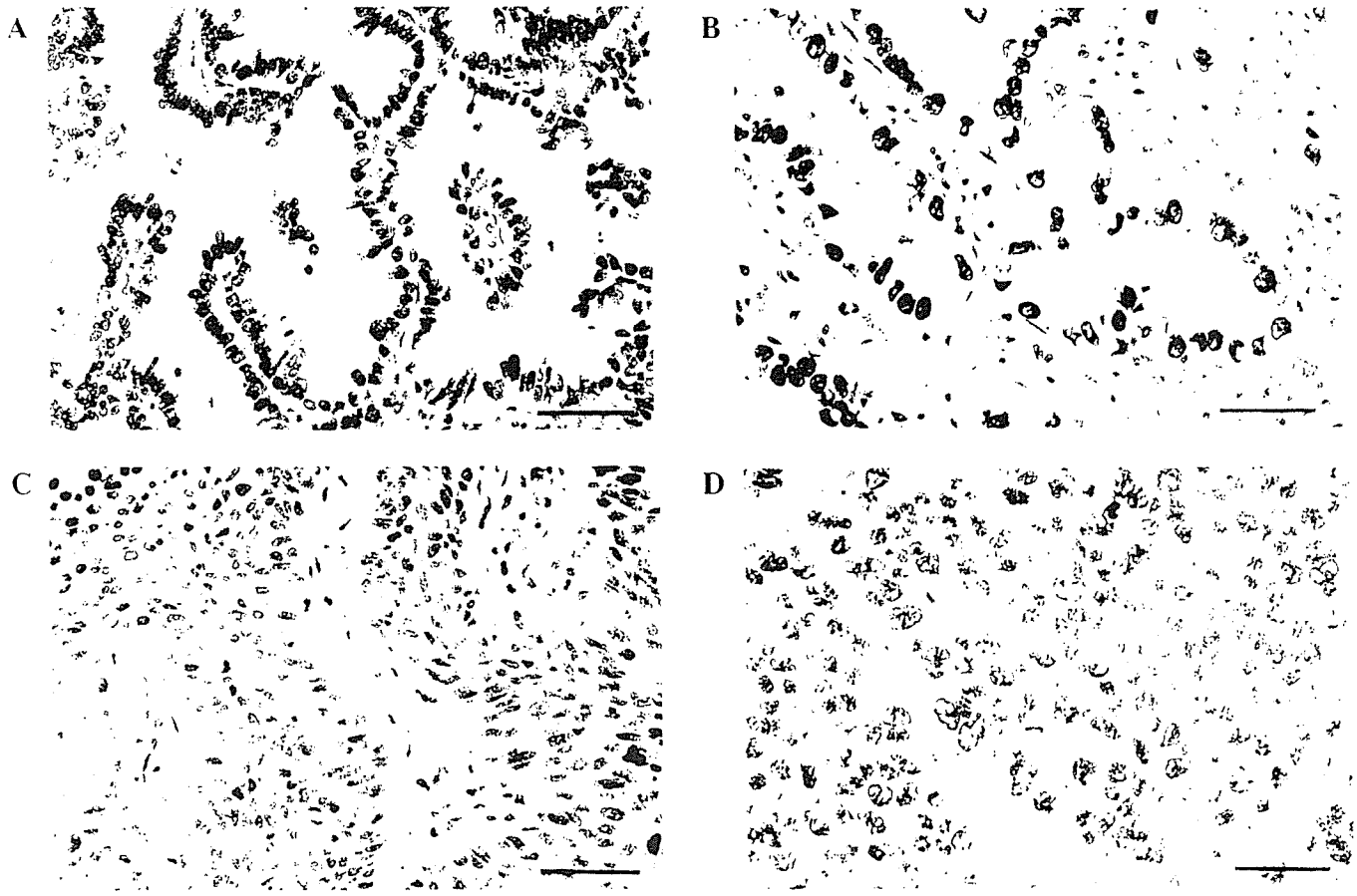

Figure 1. Representative photomicrographs of immunohistochemical staining of HDGF in adenocarcinoma (A and B) and squamous cell carcinoma (C and D) cases. HDGF is expressed weakly in the nucleus of <65% of tumor cells in A and C (defined as low HDGF-expression). To the contrary, HDGF staining was more intense in the nucleus and weak in the cytoplasm of ≥65% of tumor cells in B and D (defined as high HDGF-expression). Scale bars, 50 μm.

statistical analyses, the criterion of significance was defined as $P<0.05$.

Results

HDGF expression was detected in all tumor sections in various proportions. Many cancer cells exhibited strong HDGF-staining, mainly in the nucleus, and some cancer cells presented weak staining in the cytoplasm. Representative cases of adenocarcinoma and squamous-cell carcinoma are shown in Fig. 1. The median score of HDGF-LI in all cases was 64.5% (20-95%), and therefore we defined 65% as a cut-off for low (<65%, n=51) and high (≥65%, n=51) expression. Weak staining in the endothelial cells and smooth muscle cells in the vessels was used as the internal control as mentioned above. HDGF was also detected weakly in some of the non-cancerous type II pneumocytes and ciliated columnar epithelial cells (data not shown). These findings were consistent with those of a previous report on idiopathic pulmonary fibrosis (14).

The relationship between HDGF expression and clinicopathological variables (age, sex, smoking, tumor size, stage, T-factor, N-factor, pleural involvement, vascular involvement, lymphatic involvement, histology and differentiation) in all cases is summarized in Table I. There was no significant relationship between HDGF expression and any clinicopathological variable.

Kaplan-Meier overall and disease-free curves for HDGF expression dichotomized by the median level are shown in Figs. 2 and 3, respectively. Patients with lung cancer expressing high HDGF had a significantly worse overall and disease-free survival than those with lung cancer expressing low HDGF ($P=0.0004$ and $P=0.0005$ by the log-rank test, respectively). Among 102 patients, 25 received adjuvant therapy: radiotherapy was given to 6 patients and systemic chemotherapy including cisplatin or a combination of uracil and tegafur (UFT) was given to 19 patients. There was no significant difference in the proportion of patients who received adjuvant therapy between the 2 groups (13/51 in the low-HDGF group and 12/51 in the high-HDGF group, $P>0.99$).

In the univariate analysis of correlations between prognosis and potential prognostic factors evaluated (HDGF expression, adjuvant therapy and the 12 clinicopathological variables shown in Table I), vascular involvement, smoking, N-factor, tumor size, sex, pathological stage and HDGF were significant prognostic factors ($P<0.05$) for overall and disease-free survival. Adjuvant therapy was not a significant factor for overall ($P=0.580$) or disease-free ($P=0.536$) survival in this study. These 7 significant variables were entered into the Cox proportional-hazards model and multivariate analysis was performed (Table II). Pathological stage and HDGF-expression level were significant independent prognostic factors for overall and disease-free survival, and moreover HDGF had

Table I. Association between HDGF-expression and clinico-pathological variables in all cases.

Variables	High-HDGF (%)	Low-HDGF	P-value[a]
Age			
<70 years	37 (50.0)	37	
≥70 years	14 (50.0)	14	>0.999
Sex			
Male	38 (55.1)	31	
Female	13 (39.4)	20	0.204
Smoking			
<40 pack-year	28 (54.9)	23	
≥40 pack-year	23 (45.1)	28	0.428
Tumor size			
≤30 mm	17 (40.5)	25	
>30 mm	34 (59.5)	26	0.159
pStage[b]			
Stage I + II	30 (49.2)	31	
Stage III + IV	21 (51.2)	20	>0.999
pT-factor[b]			
T1 + T2	42 (49.4)	43	
T3 + T4	9 (52.9)	8	>0.999
pN-factor[b]			
N0	25 (51.0)	24	
N1 + N2	26 (49.0)	27	>0.999
Pleural involvement[c]			
P0 + P1	40 (49.4)	41	
P2 + P3	11 (50.6)	10	>0.999
Vascular involvement			
v (-)	19 (48.7)	20	
v (+)	32 (50.8)	31	>0.999
Lymphatic involvement			
ly (-)	16 (50.0)	16	
ly (+)	35 (50.0)	35	>0.999
Histology[d]			
Ad	38 (54.3)	32	
Sq	13 (40.6)	19	0.286
Differentiation			
Well	25 (45.5)	30	
Moderate/poor	26 (55.3)	21	0.427

[a]χ^2-test. [b]According to the AJCC/UICC TNM pathological classification. pStage, pathological stage; pT, pathological tumor; pN, pathological lymph node; [c]According to the general rules for clinical and pathological record of lung cancer established by the Japan Lung Cancer Sosiety. [d]Ad, adenocarcinoma; Sq, squamous cell carcinoma.

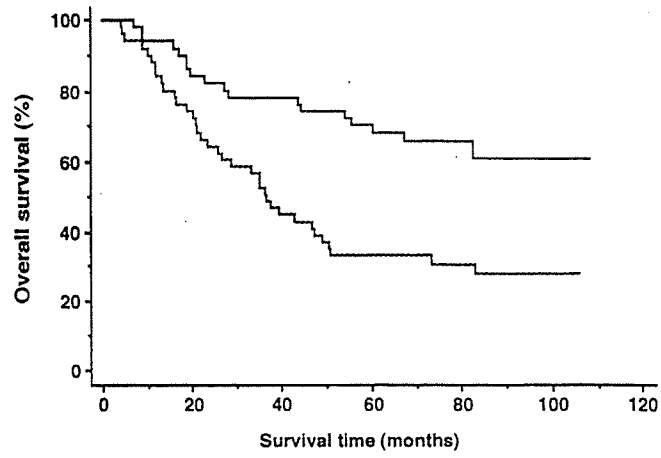

Figure 2. Kaplan-Meier analysis of the overall survival of NSCLC patients with low (a solid line) and high (a dotted line) HDGF-expression. P=0.0004 by the log-rank test.

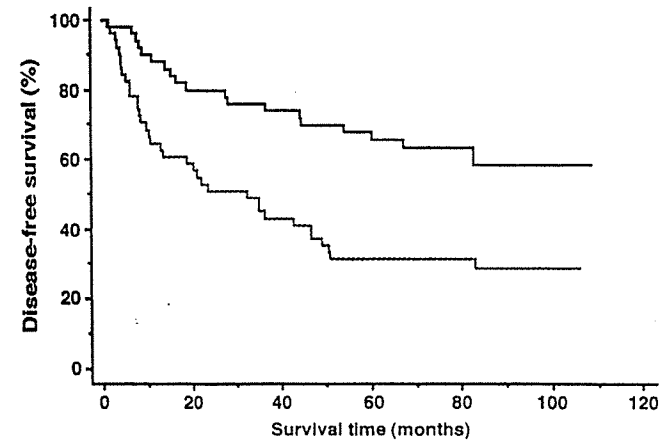

Figure 3. Kaplan-Meier analysis of the disease-free survival of NSCLC patients with low (a solid line) and high (a dotted line) HDGF-expression. P=0.0005 by the log-rank test.

higher risk-ratios than pathological stage for overall (2.976 versus 1.964) and disease-free survivals (2.970 versus 1.848).

The role of HDGF in the biological behavior of NSCLC remains to be fully elucidated. HDGF was very recently reported to be not only a mitogenic factor for lung epithelial cells but also an angiogenic factor (6,9,10,14). We, therefore, examined the relationship between HDGF-expression level and Ki-67-LI or MVD in serial sections. Ki-67-LI values for low and high HDGF-expressing cases were 19.3±14.3% and 34.1±17.7%, respectively (mean ± SD, P<0.00005 by Student's t-test). MVD values for low and high HDGF-expressing cases were 49.8±23.6 and 68.5±20.7 vessels/mm^2, respectively (mean ± SD, P<0.00005 by Student's t-test). These findings suggested that HDGF may promote the proliferation of tumor cells and intratumor angiogenesis in lung cancer.

Discussion

We demonstrated that HDGF is mainly expressed in the nucleus of NSCLC cells and that high expression of HDGF

Table II. Multivariate analysis of prognostic factors.

Variables	Unfav./Fav.[a]	Overall survival Risk ratio (95% CI[b])	P-value	Disease-free survival Risk ratio (95% CI)	P-value
Vascular involvement	v (+)/v (-)	1.016 (0.512-2.014)	0.9644	1.044 (0.532-2.048)	0.9009
Smoking	≥40/<40 pack-year	1.398 (0.700-2.792)	0.3427	1.632 (0.815-3.270)	0.1667
pN-factor[c]	N1 + N2/N0	1.462 (0.760-2.811)	0.2548	1.246 (0.649-2.392)	0.5083
Tumor size	>30/≤30 mm	1.572 (0.853-2.895)	0.1468	1.443 (0.782-2.663)	0.2404
Sex	Male/female	1.957 (0.856-4.472)	0.1114	1.956 (0.860-4.450)	0.1096
pStage[c]	III + IV/I + II	1.964 (1.084-3.556)	0.0259	1.848 (1.018-3.355)	0.0436
HDGF	High/low	2.976 (1.641-5.398)	0.0003	2.970 (1.651-5.344)	0.0003

[a]Unfavorable vs. favorable characteristics. [b]CI, confidence interval. [c]According to the AJCC/UICC TNM pathological classification. pStage, pathological stage; pN, pathological lymph node.

is an independent significant factor for worse overall and disease-free survival of patients with completely resected NSCLC. We also showed that HDGF-expression level was associated with both a high Ki-67-LI and a high intratumor MVD.

Recently, Ren *et al* (23) reported that overexpression of HDGF was a marker of poor prognosis only in patients with curatively resected stage I NSCLC. They found no association between HDGF expression and Ki-67-LI of cancer cells, which is inconsistent with our results. This difference may be because our study included stage I-IV cases whereas theirs only included stage I cases: HDGF-expression correlated with Ki-67-LI in stage IB-IV but not in stage IA (data not shown). Thus, we demonstrated that HDGF-expression level is a prognostic factor independent of and more powerful than the pathological stage of NSCLC by the multivariate analysis.

Exogenous HDGF promotes *in vitro* DNA synthesis and cell proliferation in rat and human lung epithelial cells. Endogenous HDGF overexpressed via transient gene transfer was translocated into the nucleus and promoted the proliferation of human lung epithelial A549 cells. Mori *et al* (14) confirmed, using short interfering RNA technique, that endogenously produced HDGF has a mitogenic effect on A549 cells. Collectively, HDGF probably stimulates the proliferation of lung epithelial cells, at least partially, in an autocrine manner. These findings support our result that high expression of HDGF correlates with a high Ki-67-LI of cancer cells. To date it is unknown if the exogenous mitogenic effect of HDGF is mediated by a cell surface receptor or uptake of the protein (4). Further exploration of this mechanism will contribute to the precise understanding of the biological functions of HDGF.

HDGF induced tumorigenesis of NIH3T3 cells in nude mice via direct angiogenic activity and induction of VEGF (7). Moreover, HDGF is a highly expressed vascular endothelial cell protein *in vivo* and is a potent endothelial mitogen and regulator of endothelial cell migration that acts through mechanisms distinct from those of VEGF (6). Since we could not observe a remarkable enhancement of HDGF expression in endothelial cells or vascular smooth muscle cells in NSCLC sections (data not shown), overproduction of HDGF by cancer cells might induce a high intratumor MVD possibly in a paracrine manner.

HDGF shows a homology to high mobility group-1 (HMG-1), a DNA binding protein (24), but lacks the characteristics of an HMG-1 protein, especially of the HMG box responsible for DNA bindings (2). HMG-1 enhances the activity of several transcription factors, including the glucocorticoid receptor, as well as the activity of RAG recombinase (24,25). The molecules controlled by HDGF in the nucleus and subsequent functions of HDGF have not been identified. Therefore, HDGF may display other tumorigenic behavior besides tumor-cell proliferation or angiogenesis.

Bernard *et al* (26) detected HDGF expression mainly in the nucleus and much more strongly in melanoma cell lines than in melanocytes. They showed by immunohistochemical analysis of clinical samples that 54% of benign nevoid cells reacted positively, whereas 78-90% of melanoma cells were positive in all stages of melanoma. We found that HDGF was expressed in ~30-40% of non-cancerous alveolar or bronchial epithelial cells (data not shown) and 20-95% (median 64.5%) of NSCLC cells. The proportion of HDGF-positive cells seems slightly smaller in NSCLC than in melanoma, but our results were in general compatible with those of Bernard *et al* (26). With regard to other malignancies, a recent study using differential display revealed that HDGF expression was associated with radiosensitivity in esophageal cancer (27). Expression profiling of gastric adenocarcinoma using cDNA array revealed that HDGF was one of the overexpressed genes in gastric cancer as compared with normal gastric mucosa (28). Thus, HDGF probably plays a critical role in the development and progression of various malignancies.

Based on the above findings, we consider HDGF is a useful marker of poor prognosis in patients with completely resected NSCLC, and high HDGF-expression might be a potential indicator of the need for adjuvant therapy. Although further investigations need to be done on the molecular characteristics and biological functions of HDGF, this factor may be a target molecule for the treatment of NSCLC.

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Retrospective analysis of the predictive factors associated with the response and survival benefit of gefitinib in patients with advanced non-small-cell lung cancer

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Prognostic factor;
Smoking index;
Female;
Performance status (PS);
Retrospective analysis

Summary

Background: The purpose of the study was to identify the potential predictive features associated with the response and survival benefit of gefitinib administration. We have retrospectively reviewed data of all patients who received a single regimen of gefitinib in our institution from August 1998 until July 2003.

Methods: Overall 101 patients with non-small-cell lung cancer (NSCLC) who have received a single use of gefitinib were analyzed. Potential factors associated with the response of gefitinib included smoking index, gender, histology, performance status (PS), number of pre-treatments, age and stage. Univariate analysis was performed for these strata by Fisher's exact test and multivariate analysis was then performed using the logistic regression model.

Results: The overall response rate was 19.8%. Univariate analysis revealed that significant predictive factors were associated with the response for 'adenocarcinoma', 'female', 'good PS' (0–l) and 'non-smoker' categories. Multivariate analysis limited the predictive factors associated with the response for 'female' ($P = 0.0032$), 'good PS' ($P < 0.02$) and 'non-smoker' ($P = 0.0417$). In survival analyses, 'female' ($P < 0.005$), 'good PS' ($P < 0.0001$), and a low level of the smoking index ($P < 0.05$) indicated significantly prolonged survival. Response and survival data in elderly patients were equivalent to those in younger patients. Adverse events (AEs) were generally mild and were almost always skin reactions and diarrhea. Interstitial lung disease (ILD) occurred in 4% of the group under observation.

Conclusions: Gefitinib provided clinical benefit for the following factors 'female', 'good PS' and 'non-smoker'. A low smoking index is reported as a novel predictive prognostic factor following a single regimen of gefitinib.

Abbreviations: NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor; IDEAL-1, Iressa dose evaluated advanced lung cancer-1; PS, performance status; NCI-CTC, National Cancer Institute-Common Toxicity Criteria; INTACT-1, Iressa NSCLC trial assessing combination treatment-1; INTACT-2, Iressa NSCLC trial assessing combination treatment-2

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1. Introduction

Patients with advanced non-small-cell lung cancer (NSCLC) have a poor prognosis with 1–5% 5-year survival rates [1]. A recent meta-analysis demonstrated that platinum-based combination chemotherapy is currently considered to be the most effective treatment for advanced NSCLC, and these have improved the median survival time (MST) by 2 months and caused a 10% increase in 1-year survival rates [2]. As platinum-based chemotherapy improves survival and quality of life in advanced NSCLC patients, most patients will receive second line chemotherapy. With recurrence or progression, docetaxel has been approved as a second line chemotherapy treatment due to demonstrated survival benefit compared with best supportive care (BSC) or vinorelbine/ifosfamide [3,4]. Currently, there is no proven effective chemotherapy for patients previously treated with platinum-based and docetaxel therapies.

The epidermal growth factor receptor (EGFR) is a promising target for anticancer therapy because many types of cancer cells express or overexpress EGFR (including NSCLC, renal cell carcinoma and breast cancer) [5,6]. EGFR overexpression has been reported as a poor prognostic factor in many types of human solid tumors including NSCLC in several studies [7–9]. Currently, monoclonal antibodies that bind to the extracellular domain of EGFR and intracellular tyrosine kinase inhibitors have been developed [10,11]. Gefitinib is an orally active, selective EGFR tyrosine kinase inhibitor that blocks signal transduction pathways implicated in the proliferation, angiogenesis, invasion, metastasis and survival of cancer cells [12,13]. Several phase I trials demonstrated safety and tolerability of gefitinib in pretreated patients with solid tumors, in which trials an 11% response rate was seen in 100 patients with heavily pretreated advanced NSCLC [14]. On the other hand, in Japan, a phase I trial demonstrated five responders out of a total of 31 patients who all had adenocarcinoma of the lung [12]. To confirm anti-tumour activity and the safety profile of gefitinib, an international phase II study (IDEAL-1) and United States trial (IDEAL-2) were conducted as a second or third line treatment in patients with advanced NSCLC [15,16]. Patients enrolled in these studies were randomized into two different doses, 250 and 500 mg/day. These trials demonstrated that toxicity was mild and showed an encouraging response rate with an RR of 18.4 and 11.8% of patients in the 250 mg arm, respectively, and an improvement in disease related symptoms and quality of life were observed. The IDEAL-1 study has also confirmed that there were statistically significant differences in efficacy for 'adenocarcinoma' and 'female' using multivariate analysis. Two large randomized phase III studies [17,18], which are standard chemotherapy (cisplatin/gemcitabine or carboplatin/paclitaxel) with or without gefitinib, failed to demonstrate a survival benefit for advanced NSCLC patients as a first line chemotherapy. Although the results of the phase III studies were negative, gefitinib is still considered a promising molecular targeted agent as a new generation treatment in patients with advanced NSCLC. Information on the clinical prognostic factors following a single regimen of gefitinib should be helpful in finding which patients are likely to receive benefit, and in the development of a future treatment. Although the previous phase II trial (IDEAL) showed that several predictive factors were associated with the response to gefitinib, the population was essentially biased towards the young, with good performance status (PS) and conserved, good organ functions.

In this study, to find factors associated with an objective response and survival benefit of gefitinib, we retrospectively analysed patients who received a single regimen of gefitinib at our institute.

2. Methods

All patients with stage IIIB or IV NSCLC, who received a single regimen of gefitinib from August 1998 until July 2003 at the Kinki University School of Medicine, Osaka, were retrospectively reviewed. We evaluated patients who participated in clinical trials (phase I trial, phase II trial; IDEAL-1), or phase II trial for investigating surrogate gene therapy, and in 53 patients who were administered the drug after marketing (including elderly or poor performance status patients). Patients who received gefitinib as part of a compassionate use program were excluded. All patients were checked for age, gender, histology, Eastern Cooperative Oncology Group (ECOG), PS, stage, pre-treatment regimen, number of prior regimen, and smoking status before treatment of gefitinib. Smoking status was evaluated by the Brinkmann index; number of cigarettes per day multiplied by number of years. We analyzed the response, overall survival rate and the adverse effects of gefitinib, and investigated predictive factors associated with response and prognosis. The response was assessed using physical examination, biochemical profile, chest X-ray, chest computed tomography (CT), head CT or magnetic resonance imaging (MRI) scan, abdominal echo-graphic or abdominal CT scan, bone scinti-graph, bronchoscope, and was evaluated according to the response eval-

uation criteria in solid tumor (RECIST) [19]. The severity of all the adverse events (AEs) that related to gefitinib administration was assessed by the NCPCTC (version 2.0) grading system. The predictive factors associated with the response that were analyzed in this study were age, gender, PS, histology, stage, number of prior regimen and smoking status. Variables were tested for any possible relationship with the response to gefitinib, at first by univariate analysis, and subsequently by the application of a multivariate model. Response rates were compared between strata using Fisher's exact test. Logistic regression models were used to explore observed differences and identify baseline factors that may independently predict for response rates. The survival curves were estimated using the Kaplan–Meier method and compared using the log-rank test. *P*-values less than 0.05 were considered significant.

3. Results

3.1. Patient profiles

From August 1998 until July 2003 at our institute, a total of 105 patients, who were already cytologically or histologically diagnosed as NSCLC, were treated by a single regimen of gefitinib. Patients received gefitinib until disease progression or intolerable toxicity. Of these, 101 patients were evaluated as suitable for analysis; four patients were excluded from analysis because they received gefitinib as part of a compassionate use program. As shown in Table 1, the 101 patients included: 2 patients who received gefitinib at a once daily dose of 50 mg; single patients who each received 100, 225, 400, 525 and 700 mg, respectively; 89 patients who received 250 mg; and 5 patients who received 500 mg. In the phase I trial, we used an intermittent administration schedule with 14 days continuous dosing followed by 14 days off.

Table 1 Patient characteristics

	Number of patient ($N = 101$)
Phase I	7
50 mg	2
100 mg	1
225 mg	1
400 mg	1
525 mg	1
700 mg	1
Phase II (IDEAL-1)	11
250 mg	6
500 mg	5
Phase II (gene expression) (250 mg)	30
Post marketing (250 mg)	53

Table 2 Patient characteristics ($N = 101$)

	Number of patients
Age (year)	
Median (range)	62 (31–84)
<69	74
≥70	27
Gender	
Male	64
Female	37
Performance status	
0	15
1	62
2	17
3	7
Tumor histology	
Adenocarcinoma	81
Squamous	18
Large-cell	2
Stage	
III	18
IV	83
Previous treatment	
No treatment	5
Failed 1 previous chemotherapy regimens	53
Failed 2 previous chemotherapy regimens	34
Failed 3 previous chemotherapy regimens	9
Smoking (smoker:never-smoker)	55:46
Index[a] 0:1–999:1000	46:32:23

[a] Index: number of cigarettes per day multiplied by number of years.

Patient characteristics are shown in Table 2. The median age was 62 years (ranging from 31–84) and 74 patients (73.3%) were less than 69 years old. 63.4% of the patients were male, 76.2% had performance status (ECOG) 0–1, 80.2% had adenocarcinoma of which 83.2% had stage IV disease. Fifty-three patients had received one prior regimen, 43 had more than two prior regimens and only five had previously been untreated. 54.5% of them were smokers, and the non-smokers were almost all female. This study included patients

Table 3 Overall objective response

	Number	%
Number of patients evaluated	101	
Complete response (CR)	1	1.0
Partial response (PR)	19	18.8
Stable disease (SD)	52	51.5
Progressive disease (PD)	25	24.8
Not evaluable	4	4.0
Response rate % (95% CI)	19.8 (12.0–27.6)	
Disease control rate[a] % (95% CI)	71.3 (62.5–80.1)	

[a] CR + PR + S.D.

who had failed several previous chemotherapy regimens, and patients with an ECOG PS score of 3.

3.2. Response to treatment

Table 3 shows an objective response observed in this study. Twenty responders were evaluated and the overall response rate was 19.8%. One patient achieved a complete response, 19 patients exhibited a partial response and 52 patients had stable disease, resulting in a disease control rate (objective responses plus stable disease) of 71.3%. When evaluated using patient characteristics, we determined the response rate detailed in Fig. 1. All patients that responded had adenocarcinoma of the lung as the histological subtype. In addition, for the factors 'female' and 'never-smoker', there were higher response rates than in 'male' and 'smoker' respectively, while RR was similar for age, stage and pre-treatment. The response rate of 'female' and 'never-smoker' were 37.8 and 32.6%, respectively. Using the Fisher's exact test, the predictive factors which were associated with a response were 'female' (37.8% versus 9.4%; $P = 0.0006$), 'adenocarcinoma' (24.7% versus 0%; $P = 0.0104$), 'good PS' (0–1) (26.0% versus 0%; $P = 0.0028$), and never-smoker (32.6% versus 9.1%; $P = 0.0025$). There were no significant differences for age, stage and pre-treatment (Table 4). A multivariate analysis was performed against the four significant predictive factors in univariate analysis (Table 5). Because the incidence of the female factor is very strongly correlated to the never-smoker factor, the statistical assay was rather unstable if the two factors were analyzed simultaneously. We then investigated two patterns of multivariate analysis. One analysis excluded smoking and the other excluded gender. If smoking status was extracted, then female and good performance status were statistically significant. If gender was extracted, then non-smoking and good performance were statistically significant. The odds of a response were over three times higher for patients with adenocarcinoma than for patients with other histologies, however, this is not considered to be statistically significant because the group in this study was of a small size and included a high percentage of adenocarcinoma.

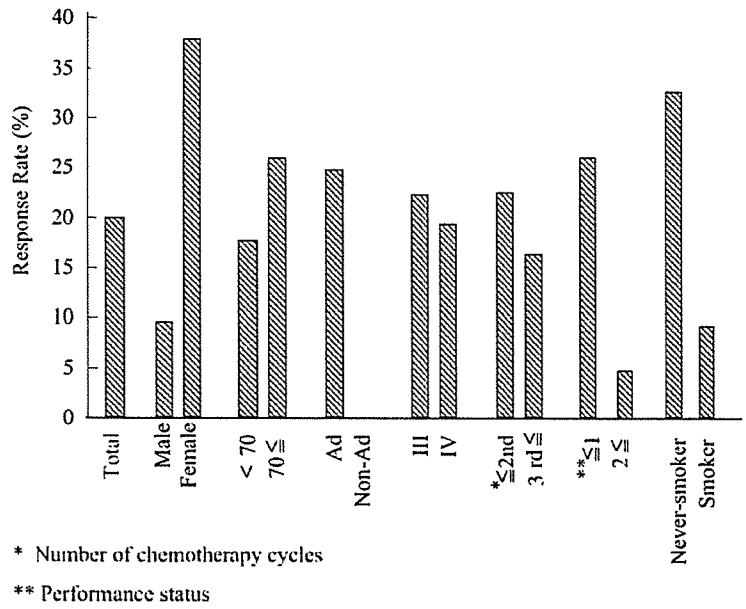

Fig. 1 Tumor response rate of the subgroups.

Table 4 Predictive factors associated with an objective response by univariate analysis

Parameter	*N*	Responder	RR (%)	*P*-value
Smoking index				
Non-smoker	55	15	32.6	
Smoker	46	5	9.1	0.0025
Gender				
Female	37	14	37.8	
Male	64	6	9.4	0.0006
Histology				
Adenocarcinoma	81	20	24.7	
Others	20	0	0.0	0.0104
PS				
0–1	77	20	26.0	
≥2	24	0	0.0	0.0028
Pre-treatment				
≤2 regimens	58	13	22.4	
≥3 regimens	43	7	16.3	N.S.
Age (years)				
≤70	74	13	17.6	
≥71	27	7	25.9	N.S.
Stage				
IIIB	18	4	22.2	
IV	83	16	19.3	N.S.

Abbreviations: N.S., not significant.

3.3. Toxicity

Drug-related AEs of all patients are shown in (Table 6). A total of 101 patients were evaluated for toxicity. The most frequent drug-related AEs were a rash, dry skin and diarrhea. Most of these AEs were mild (Grade 1 or Grade 2) and were controllable. Of all the drug-related AEs evaluated, Grade 3 or Grade 4 AEs were seen in less than 5%, and Grade 4 drug-related AEs were only pneumonitis. Grade 3 or 4 AEs required a treatment interruption, but recovered after discontinuation of gefitinib, except with pneumonitis. Four patients developed greater than Grade 3 pneumonitis requiring hospitalization. All patients had a fever and severe hypoxemia on admission. As soon as possible, all patients were administered steroid therapy. While two patients recovered with the steroid therapy, two patients died within 40 days after the administration of gefitinib. Hematological toxicities were not observed.

3.4. Survival

The median survival time of the patients who were 'good PS' (0 or I) and 'poor PS' (2 or 3) was 353 and 97 days, respectively, and this difference was significant ($P = 0.0001$, log-rank test) (Fig. 2A). The MST of females was significantly longer than that of males (596 days versus 178 days, $P = 0.004$) (Fig. 2B). Furthermore, a low smoking index (<900) significantly prolonged survival (MST: 301 days versus 149 days, $P = 0.031$) (Fig. 2C). Age did not influence the survival benefit of the patients treated with gefitinib (Fig. 2D).

4. Discussion

Gefitinib is an orally active, selective EGFR tyrosine kinase inhibitor that blocks signal transduction pathways, and is one of the promising molecular targeted drugs used in the treatment of advanced NSCLC [16,17,20]. Although the large scale of the phase II study (IDEAL-1) [15] has already confirmed that there were statistically significant differences in efficacy for 'adenocarcinoma' and 'female' by multivariate analysis, the population was essentially biased towards young people with good performance status who had conserved, good organ functions. To clarify the predictive prognostic fac-

Table 5 Predictive factors associated with an objective response by multivariate analysis

Parameter	Odds ratio	95% CI	*P*-value
Extraction of smoking			
Gender (female vs. male)	0.163	0.040–0.585	0.0032
Performance status (1 vs. 2)	0.061	0.000–0.415	0.0018
Histology (Adeno[a] vs. others)	3.326	0.435–infinity	N.S.
Extraction of gender			
Non-smoking (non vs. ≥1)	0.297	0.063–0.959	0.0417
Performance status (1 vs. 2)	0.096	0.000–0.628	0.0101
Histology (Adeno vs. others)	4.385	0.588–infinity	N.S.

Abbreviations: N.S., not significant; CI, confidence interval.
[a] Adenocarcinoma.

Table 6 Patients with drug-related adverse events (NCI-CTC)

Adverse event	Number of patients ($N = 101$)				
	Grade 1	Grade 2	Grade 3	Grade 4/5	Total
Rash	33 (32.6%)	21 (20.8%)	3 (3.0%)	0	57 (56.4%)
Dry skin	24 (23.7%)	3 (3.0%)	0	0	27 (26.7%)
Pruritis	9 (9.0%)	7 (7.0%)	0	0	16 (16.0%)
Diarrhea	19 (18.8%)	4 (4.0%)	0	0	23 (22.8%)
Nausea	6 (6.0%)	1 (1.0%)	0	0	7 (7.0%)
Vomiting	3 (3.0%)	0	0	0	3 (3.0%)
Anorexia	7 (7.0%)	0	0	0	7 (7.0%)
ALT increased	5 (5.0%)	2 (2.0%)	5 (5.0%)	0	12(13.0%)
AST increased	8 (8.0%)	2 (2.0%)	3 (3.0%)	0	13 (13.0%)
Pneumonitis	0	0	2 (2.0%)	2[a] (2.0%)	4 (4.0%)

[a] Treatment-related death (Grade 5).

tors in a practical setting, we retrospectively analysed the patients who received a single regimen of gefitinib at our institute. Multivariate analysis demonstrated that the predictive factors which were associated with a response were 'female', 'good PS' and 'never-smoker'. In survival analyses, the factors 'female', 'good PS', and a low smoking index also significantly prolonged survival.

The mechanism by which these factors produced better prognosis has not been clarified.

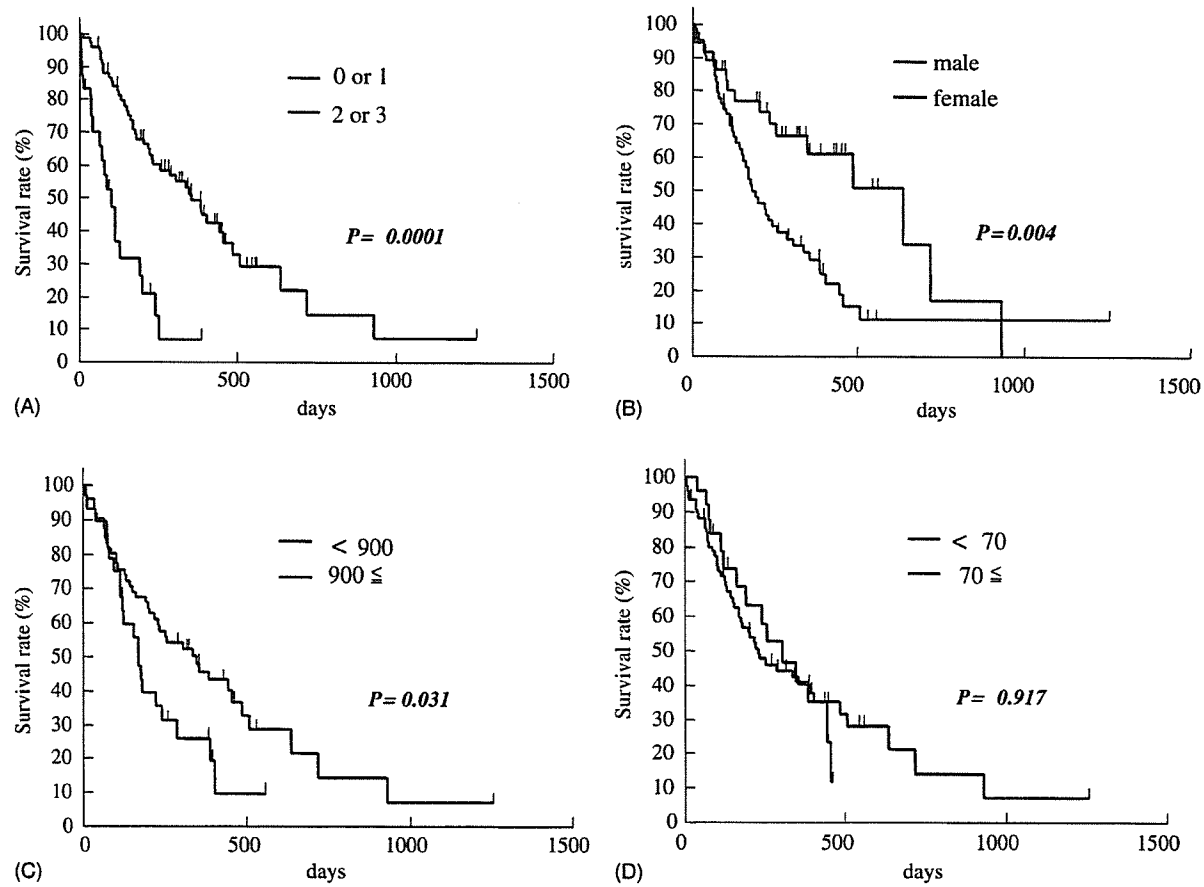

Fig. 2 A comparison of survival of: (A) PS 0, 1 vs. PS 2, 3; (B) gender: male vs. female; (C) smoking index: <900 vs. ≥900; and (D) age: <70 vs. ≥70.

Estrogen and progesterone may up-regulate EGFR in normal tissues [21], and activation of steroid hormones might impact on EGFR function in NSCLC [22]. Another explanation may be that the steroid hormone receptor might interact with EGFR and influence the response of an EGFR inhibitor.

Multivariate analysis in IDEAL-1 showed that PS was not a significant prognostic factor, however, the population of the study was restricted with regards to good PS. Although gefitinib was considered as an effector of symptom improvement in the phase II trial, the indication for patients with poor PS is controversial. Several authors described the case reports about the efficacy of gefitinib in NSCLC patients with poor PS [23,24] or with brain metastases [25]. Although 'good PS' were significant prognostic factor in this trial, gefitinib still might be a candidate drug for patients with poor PS, because of restriction of the use of other anti-cancer drug by their toxicities.

Elderly patients exhibited an equivalent response to young patients in this study. Recent data suggested, gefitinib is safe and well tolerated in elderly pretreated NSCLC patients [26]. A phase II study of gefitinib for elderly patients in NSCLC is needed.

A low smoking index was revealed as a predictive prognostic factor following a single regimen of gefitinib. Erlotinib is also administered orally and is a highly selective EGFR tyrosine kinase inhibitor [27] with a quinazolinamine-based structure similar to that of gefitinib. In the phase II study of erlotinib in NSCLC or bronchial alveolar carcinoma [28], a non-smoking history was also a prognostic factor. Chronic exposure to nicotine increases the expression level and phosphorylation status of EGFR and impairs its function [29]. Moreover, smoking produces overexpression of Her2/neu that binds to EGFR as a hetero-dimer in the tissue of normal bronchus. Expression of EGFR or Her2/neu or both in tissue samples by immunohistochemistry has not correlated in the response of gefitinib [30], however the different type of dimers formed between EGFR families might influence the response to gefitinib.

Four patients (4% of the patients) developed interstitial lung disease (ILD). Continuous smoking disrupted surfactant protein A or D [31,32], and the serum levels of the proteins were increased [33]. As 'smoking history' and 'male' are significant risk factors of ILD and also in treatment with gefitinib [34], a serum level of the surfactant protein A or D might be a predictive marker of ILD. Patients who are female and non-smokers are most likely to receive a high benefit and low risk with gefitinib treatment.

Although more basic biological research is needed to find the mechanism of action, we have found several predictive prognostic factors associated with the practical use of gefitinib. This is necessary clinical information which is important in order to set eligibility criteria for future clinical trials with gefitinib.

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Combination phase I study of nedaplatin and gemcitabine for advanced non-small-cell lung cancer

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To establish the toxicities and maximum tolerated dose (MTD) of nedaplatin with gemcitabine, and to observe their antitumour activity, we conducted a combination phase I study in advanced non-small-cell lung cancer (NSCLC). Patients received nedaplatin (60–100 mg m^{-2} given intravenously over 90 min) on day 1, and gemcitabine (800–1000 mg m^{-2} given intravenously over 30 min) on days 1, 8, every 3 weeks. In total, 20 patients with locally advanced or metastatic NSCLC who received no prior chemotherapy or one previous chemotherapy regimen were enrolled. The most frequent toxicities were neutropenia and thrombocytopenia; nonhaematological toxicities were generally mild. Three out of six patients experienced dose-limiting toxicities (neutropenia, thrombocytopenia and delayed anaemia) at dose level 4, 100 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine, which was regarded as the MTD. There were three partial responses, for an overall response rate of 16.7%. The median survival time and 1-year survival rate were 9.1 months and 34.1%, respectively. This combination is well tolerated and active for advanced NSCLC. The recommended dose is 80 mg m^{-2} nedaplatin with 1000 mg m^{-2} gemcitabine. This combination chemotherapy warrants a phase II study and further evaluation in prospective randomised trials with cisplatin- or carboplatin-based combinations as first-line chemotherapy for advanced NSCLC.

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Based on the results of a meta-analysis (Non-Small Cell Lung Cancer Collaborative Group, 1995), cisplatin-based chemotherapy is considered the best available therapy for patients with locally advanced or metastatic non-small-cell lung cancer (NSCLC). Although several new agents with novel mechanisms and significant activity against NSCLC have been introduced, such as taxanes, gemcitabine and vinorelbine, any of these agents used in combination with a platinum agent provide equivalent survival improvement (Kelly *et al*, 2001; Schiller *et al*, 2002; Fossella *et al*, 2003). The prognosis of advanced NSCLC patients who receive cisplatin-based chemotherapy is still poor, and the renal and gastrointestinal toxicities caused by cisplatin often limit its clinical use. Therefore, development of different treatment strategies is necessary.

Nedaplatin is a second-generation platinum derivative that has shown equivalent antitumour activity and lower toxicity – less nausea, and lower nephrotoxicity and neurotoxicity – than cisplatin (Kameyama *et al*, 1990; Ota *et al*, 1992). A phase I study demonstrated the maximum tolerated dose (MTD) and the recommended dose (RD) for phase II studies of nedaplatin was 120 and 100 mg m^{-2}, respectively, and the dose-limiting toxicity (DLT) was thrombocytopenia (Ota *et al*, 1992). Two independent phase II studies of nedaplatin for NSCLC showed response rates of 14.7 and 20.5%, respectively, and 16.7 and 12.5% with the patients who had received chemotherapy previously (Fukuda *et al*, 1990; Furuse *et al*, 1992a). Based on these promising results, a randomised study of nedaplatin–vindesine *vs* cisplatin–vindesine was conducted for previously untreated NSCLC patients in Japan and indicated that nedaplatin-based chemotherapy yielded similar response rates and overall survival (Furuse *et al*, 1992b). Leucopenia, renal toxicities and gastrointestinal toxicities were more frequent in the cisplatin–vindesine arm, while thrombocytopenia was more frequent in the nedaplatin–vindesine arm.

Gemcitabine, an analogue of deoxycytidine, is a pyrimidine antimetabolite, that shows a reproducible response rates of >20% with a median survival time of 9 months, offering a quality of life benefit in comparison with best supportive care (Abratt *et al*, 1994; Anderson *et al*, 1994; Gatzemeier *et al*, 1996; Anderson *et al*, 2000). The main toxicity of gemcitabine is mild-to-moderate myelosuppression. The combination of gemcitabine and cisplatin showed synergistic effects in preclinical studies because gemcitabine inhibited the repair of DNA damage caused by cisplatin (Bergman *et al*, 1996), and achieved high response rates along with improvements in median survival time in clinical setting (Sandler *et al*, 2000; Schiller *et al*, 2002; Alberola *et al*, 2003).

Recently, carboplatin has attracted attention ahead of nedaplatin because it has similar activity to cisplatin with fewer nonhaematological toxicities. The available data suggest that carboplatin–paclitaxel or carboplatin–gemcitabine should be considered among standard regimen for advanced NSCLC (Kelly *et al*, 2001; Grigorescu *et al*, 2002; Rudd *et al*, 2002; Schiller *et al*, 2002).

It seems that nedaplatin has activity and toxicity profiles similar to those of carboplatin, although no randomised trial has not been done to allow direct comparison (Fukuda *et al*, 1990; Furuse *et al*,

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